COMPARISON OF ANALYTICAL TEHCNIQUES TO EVALUATE THE EFFECT OF DRYING ON OXIDATIVE STATUS OF SLICED ALMONDS DURING STORAGE

by

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ABSTRACT OF THE THESIS

Comparison of Analytical Techniques to Evaluate the Effect of Drying on Oxidative Status of Sliced Almonds during Storage

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Almonds (Prunus amygdalus*)* are highest produced tree nuts worldwide, grown mainly in Mediterranean climate. Almond contains approximately 49% total lipids of which 12g is polyunsaturated fat, 30.9% monounsaturated fat and 3.7% saturated fat. About 90% of its total fatty acid composition is unsaturated, where oleic acid is 60-70% and linoleic acid 14-26%. The unsaturated fatty acids makes the almonds susceptible to oxidative degradation by producing volatile compounds, off flavor and odor decreasing their nutritional value. The deterioration rate of the almonds lipid fraction during storage due to the oxidative process is of a great economic and nutritional importance. Therefore, before storage the fruit is subjected to multi-processing steps in order to improve its quality in term of storage stability. In this connection, the drying step and storage conditions are of decisive importance.

The drying step involves removal of moisture to prevent hydrolysis and enzymatic degradation during storage which cause split of triglyceride into glycerol and free fatty acid. However over drying or under drying could lead to oxidative degradation. The

combination of various analytical techniques is recommended to evaluate oxidative status as no single method is available to analyze both primary and secondary oxidation products together. In this connection, this project aims at providing most effective analytical technique that evaluates the efficiency of the drying conditions on the storage stability of sliced almonds. Therefore, four different analytical techniques have been selected, namely: Iodometric titration for peroxide value, titration method for Free Fatty acid (Acid Value), UV spectrophotometry for conjugated diene and triene and GC/MS for semi-volatile and volatile compounds were used.

This study shows a positive effect of the used drying conditions on the oxidative stability since the concentration of peroxides and conjugated dienes of dried almonds decreased as compared to those of non dried and ambient dried. A constant curve for free fatty acids was obtained showing that drying was done effectively as no hydrolysis has been observed. The GC-MS method seems to be the most suitable method which gives a real picture of drying effect on the oxidative status of the stored sliced almonds.

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1 INTRODUCTION

Almonds are consumed worldwide and rank first in production of tree nuts. More than 1.1 million ton of almonds were grown all over the world in year 2006 out of which California produced about 63.5%, Spain 18.6 %, Italy 10.5%, Greece 4.2% and Turkey 4.0% (Sanahuja et al., 2009). California is the largest producer of almonds in the world that grows this tree nut over 400 mile area from Bakersfield to Red Bluff. About 400,000 acres of land is used by 7000 almond growers. In California different varieties of almonds are grown which include Nonpareil, Neplus Ultra, Mission, Peerless and California. Among these five varieties Nonpareil, Mission almonds and California are the most produce (approximately 90%) and marketed (Esfahlan et al., 2010).

Figure 1: Almonds sample showing fruits on a branch, nuts following hull removal, raw kernels and sliced kernels. (Socias I Company et al., 2008)

Almonds are considered to be nutritious having certain health benefits. They show several physiological benefits because of their bioactive compounds and nutritive value (Sanahuja et al., 2011). In this context researchers have shown a decrease in LDL without having any affect on HDL by replacement of half regular fat intake with almonds (Mexis & Kontominas, 2010). Almond seed, shell and skin extracts possess potential for free radical scavenging. Moreover almonds have also shown to decrease a risk of colon cancer in rats. (Esfahlan et al., 2010). They have also shown decrease risk of diabetes because of their fatty acid composition. This fruit also possess antioxidant activity due to presence of tocopherols and polyphenols (Sanahuja et al., 2011). In addition to health benefits, almonds are also used as snacks and as ingredient in bakery, confectionary, chocolates, ice-cream and beverage processing (Mexis & Kontominas, 2010, Beltran et al., 2011). They also have application in pharmaceutical and cosmetic industry (Sanahuja et al., 2011). Therefore taking into consideration its health benefits and uses, it becomes important to know about almonds nutritional composition.

According to USDA 2011 nutritional database approximately 49% of almonds composition is lipids of which 12% is polyunsaturated fat, 30.9% monounsaturated fat and 3.7% saturated fat. About 90% of its total fatty acid composition is unsaturated, where oleic acid is 60-70% and linoleic acid 14-26% (Zacheo et al., 2000). However due to higher level of unsaturation almonds are highly susceptible to lipid oxidation. In addition to their fatty acid composition, almond undergo multi step processing such as pre-cleaning, hulling/shelling, pasteurization, blanching, slicing, drying and storage that may affect the final desirable product. Several factors affect lipid oxidation such as temperature, oxygen, fatty acid composition, water activity and irradiation. During

processing slicing, drying and storage are of utmost importance as it affects the lipid stability. While slicing almonds, the vesicles containing lipids may get damaged exposing them to oxygen, making them susceptible to oxidation. Moreover the drying and storage conditions namely temperature of drying, moisture concentration, concentration of oxygen, light exposure, temperature of storage and insect damage during post harvest or pre-harvest also have high influence on almonds quality and lipid fraction stability (Mexis et al., 2011).

The stability of almond lipid fraction during processing and storage is likely affected by chemical reactions such as hydrolysis, autoxidation, isomerization of ethylenic bonds and cyclization reactions. The lipid hydrolysis leads to the formation of fatty acids which acts as substrates of oxidation reaction. The autoxidation of lipids forms hydroperoxides that can breakdown to form aldehydes, ketones and alcohols causing off flavor and off odor. Moreover the primary and secondary oxidation products formed can react with other macronutrients and micronutrients that are responsible for loss of nutritional value and economical loss (Nejad et al., 2002). Therefore to comply with the quality standards desired by consumers, it becomes inevitable to measure oxidative status. Thus for this purpose various methods are suggested to evaluate quality of almonds.

In present study the processor invested in new infrared drying technologies where batches of fresh sliced almonds were dried. Rancidity complaints were observed in these samples that were received from consumers of large breakfast cereals manufactures. Analyses for off odor and off flavor were not accounted by peroxide value and free fatty acids. The initial complaint samples indicated oxidative rancidity responsible for off odor and off flavor. Therefore hypothesis was that heat abused with infrared drying

responsible for triggered oxidative decomposition. In this context the present study aims at comparing the different analytical methods to evaluate effect of drying on oxidative status of sliced almonds during storage. It was conducted to assist processor with adopting improved infrared drying conditions so as to predict shelf life at accelerated storage conditions.

2 LITERATURE REVIEW

2.1 Almonds Overview

2.1.1 Almonds Composition

Almonds (P. amygdalus) are grown mainly in Mediterranean climate. Their chemical and biochemical composition are the determining parameters for their quality which is governed and influenced by several factors such as cultivation, agronomics factors, climate and harvest conditions (Zacheo et al., 2000). So it is necessary to know the nutritional composition to have a better understanding about the quality. Almond consists of four parts kernel or meat, seed coat, shell and hull. Kernels are a good source of proteins, fats, minerals, fiber and vitamins. The seed coat is good source of phenolic compounds that protects from microbial contamination and oxidation (Socias I Company et al., 2008). Table 1 indicates the nutritional composition of almonds per 100gms (USDA commodity food A256, A264). Note: This nutritional values and weights are for edible portion.

Nutrient	Units	Value per 100 grams	Number of Data Points	Std. Error
Proximates				
Water	G	4.7	75	0.046
Energy	Kcal	575	0	0
Energy	kJ	2408	0	0
Protein	G	21.22	76	0.044
Total lipid (fat)	g	49.42	75	0.188
Ash	g	2.99	75	0.015
Carbohydrate, by difference	g	21.67	0	0
Fiber, total dietary	g	12.2	74	0.194
Sugars, total	g	3.89	0	0
Sucrose	g	3.6	78	0.037
Glucose (dextrose)	g	0.12	78	0.017
Fructose	g	0.09	78	0.017
Lactose	g	0	78	0
Maltose	g	0.04	78	0
Galactose	g	0.05	74	0.013
Starch	g	0.74	4	0.084
Minerals				
Calcium, Ca	mg	264	74	3.3
Iron, Fe	mg	3.72	74	0.043
Magnesium, Mg	mg	268	73	1.269
Phosphorus, P	mg	484	73	3.052
Potassium, K	mg	705	73	3.872
Sodium, Na	mg	1	68	0.164
Zinc, Zn	mg	3.08	71	0.031
Copper, Cu	mg	0.996	71	0.015
Manganese, Mn	mg	2.285	73	0.031
Selenium, Se	µg	2.5	26	0.361

Nutrient	Units	Value per 100 grams	Number of Data Points	Std. Error
Vitamins				
Vitamin C, total ascorbic acid	Mg	0	49	0
Thiamin	Mg	0.211	70	0.003
Riboflavin	Mg	1.014	73	0.025
Niacin	Mg	3.385	73	0.067
Pantothenic acid	Mg	0.469	73	0.009
Vitamin B-6	Mg	0.143	47	0.003
Folate, total	µg	50	41	2.053
Folic acid	µg	0	0	0
Folate, food	µg	50	41	2.053
Folate, DFE	mcg_DFE	50	0	0
Choline, total	Mg	52.1	0	0
Betaine	Mg	0.5	5	0.051
Vitamin B-12	µg	0	0	0
Vitamin B-12, added	µg	0	0	0
Vitamin A, RAE	mcg_RAE	0	0	0
Retinol	µg	0	0	0
Carotene, beta	µg	1	26	0.094
Carotene, alpha	µg	0	9	0
Cryptoxanthin, beta	µg	0	8	0
Vitamin A, IU	IU	1	0	0
Lycopene	µg	0	8	0
Lutein + zeaxanthin	µg	1	8	0
Vitamin E (alpha-tocopherol)	mg	26.22	75	0.178
Vitamin E, added	mg	0	0	0
Tocopherol, beta	mg	0.29	75	0.026
Tocopherol, gamma	mg	0.65	75	0.03
Tocopherol, delta	mg	0.05	74	0.007
Vitamin D (D2 + D3)	µg	0	0	0
Vitamin D	IU	0	0	0

Nutrient	Units	Value per 100 grams	Number of Data Points	Std. Error
Vitamin K (phylloquinone)	µg	0	8	0
Dihydrophylloquinone	µg	0	8	0
Lipids				
Fatty acids, total saturated		3.731	0	0
4:00	g	0	0	0
6:00	g	0	0	0
8:00	g	0	4	0
10:00	g	0	53	0
12:00	g	0	52	0
13:00	g	0	4	0
14:00	g	0.006	75	0.001
15:00	g	0	61	0
16:00	g	3.044	75	0.012
17:00	g	0.007	74	0.001
18:00	g	0.658	75	0.005
20:00	g	0.013	75	0.004
22:00	g	0.002	67	0
24:00:00	g	0	4	0
Fatty acids, total monounsaturated	g	30.889	0	0
14:01	g	0	56	0
15:01	g	0	51	0
16:1 undifferentiated	g	0.243	75	0.002
16:1 c	g	0.231	28	0.004
16:1 t	g	0.012	28	0.001
17:01	g	0.025	70	0.003
18:1 undifferentiated	g	30.611	75	0.174
18:1 c	g	30.611	33	0.32
18:1 t	g	0	33	0
20:01	g	0.01	72	0.001

Nutrient	Units	Value per 100 grams	Number of Data Points	Std. Error
22:1 undifferentiated	g	0	4	0
24:1 c	g	0	4	0
20:2 n-6 c,c	g	0.004	67	0.001
Fatty acids, total polyunsaturated	g	12.07	0	0
18:2 undifferentiated	g	12.061	75	0.078
18:2 n-6 c,c	g	12.055	46	0.119
18:2 CLAs	g	0.001	7	0.001
18:2 t not further defined	g	0.005	44	0.001
18:3 undifferentiated	g	0.006	75	0.001
18:3 n-3 c,c,c (ALA)	g	0.006	52	0.001
18:3 n-6 c,c,c	g	0	52	0
18:04	g	0	17	0
20:3 undifferentiated	g	0	56	0
20:4 undifferentiated	g	0	4	0
20:5 n-3 (EPA)	g	0	4	0
22:5 n-3 (DPA)	g	0	4	0
22:6 n-3 (DHA)	g	0	4	0
Fatty acids, total trans	g	0.017	0	0
Fatty acids, total trans-monoenoic	g	0.012	0	0
Fatty acids, total trans-polyenoic	g	0.005	0	0
Cholesterol	mg	0	0	0
Stigmasterol	mg	4	67	0.107
Campesterol	mg	5	67	0.045
Beta-sitosterol	mg	132	67	0.889
Amino acids				
Tryptophan	g	0.214	0	0
Threonine	g	0.598	0	0
Isoleucine	g	0.702	0	0
Leucine	g	1.488	0	0
Lysine	g	0.58	0	0

Nutrient	Units	Value per 100 grams	Number of Data Points	Std. Error
Methionine	g	0.151	0	0
Cystine	g	0.189	0	0
Phenylalanine	g	1.12	0	0
Tyrosine	g	0.452	0	0
Valine	g	0.817	0	0
Arginine	g	2.446	0	0
Histidine	g	0.557	0	0
Alanine	g	1.027	0	0
Aspartic acid	g	2.911	0	0
Glutamic acid	g	6.81	0	0
Glycine	g	1.469	0	0
Proline	g	1.032	0	0
Serine	g	0.948	0	0
Other				
Alcohol, ethyl	g	0	0	0
Caffeine	mg	0	0	0
Theobromine	mg	0	0	0

Table 1: Showing nutritional composition of nuts, almonds per 100gms (USDA commodity food A256, A264). Adapted from USDA Nutrient data base 2011

The kernel is a potential source of lipids, which constitute nearly 50% of kernel's composition. The fatty acid composition of this fraction is similar to that of olive oil showing several health benefits (Socias I Company et al., 2008). Of almonds total fatty acids 90 % are unsaturated, where the main components are oleic acid (60-70%) and linoleic acid (14-26%) (Zacheo et al., 2000). Though they have high nutritional value, the unsaturated fatty acids are vulnerable to oxidation which affects the stability of the lipid fraction and consequently the quality of the whole almonds (Senesi et al., 1996).

The higher the amount of unsaturated fatty acid, greater is the vulnerability to oxidation. The degradation of the unsaturated fatty acids leads to the formation of undesirable primary and secondary oxidation products such as peroxides, ketones, alcohols, aldehydes and short fatty acids (e.g. butyric acid) that gives off flavor and off odor affecting negatively the stability and the almonds quality (Sung & Jeng, 1994). Therefore, optimizing the processing condition of almonds to enhance their storage stability is still a challenging problem for the food industry.

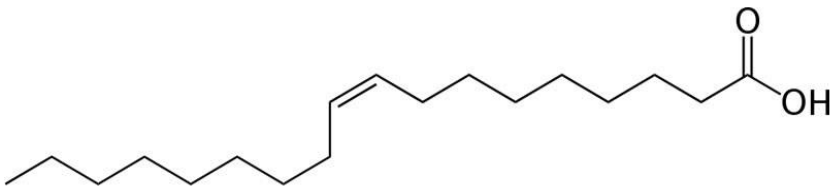

Figure 2: Structure of oleic acid

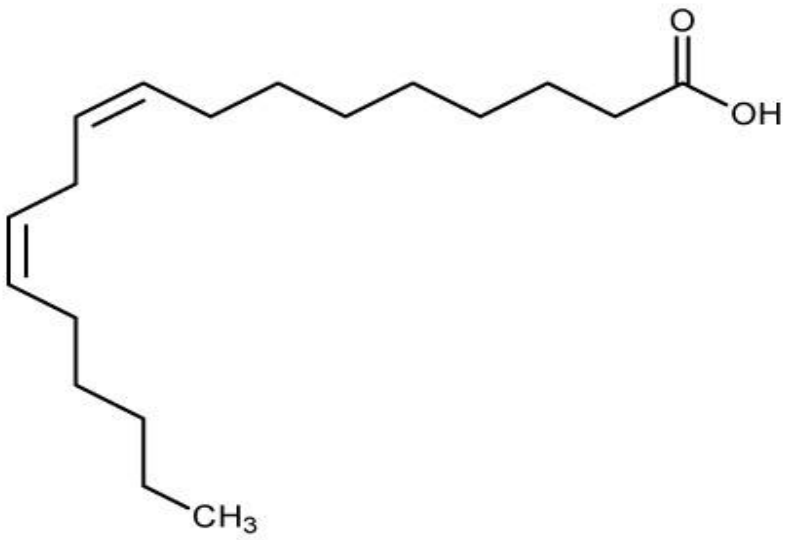

Figure 3: Structure of linoleic acid

2.1.2 Processing

The processing of almonds plays a vital role in determining shelf life of almonds. Processing involves several steps such as, precleaning, pasteurization, drying and storage. Steps such as cutting, slicing and dicing increases oxidation by exposing more surface area to oxygen. Even during pasteurizing the use of high temperature roasting can led to oxidation. Therefore critical analysis of each processing step should be done. The final processing of almonds varies for each industry in terms of different methods of pasteurization, drying, whether to slice or not, etc. But the general processing remains the same for each company such as removal of debris, precleaning, etc. Harvesting of almonds may be done manually or mechanically. They are left to dry for 7-10 days and then brought to precleaning facility where orchard debris such as leaves, stone, stick and dirt are removed. After almonds are cleaned they are hulled/shelled and brought to processing area. During processing almonds are pasteurized, sliced and dried.

Processing

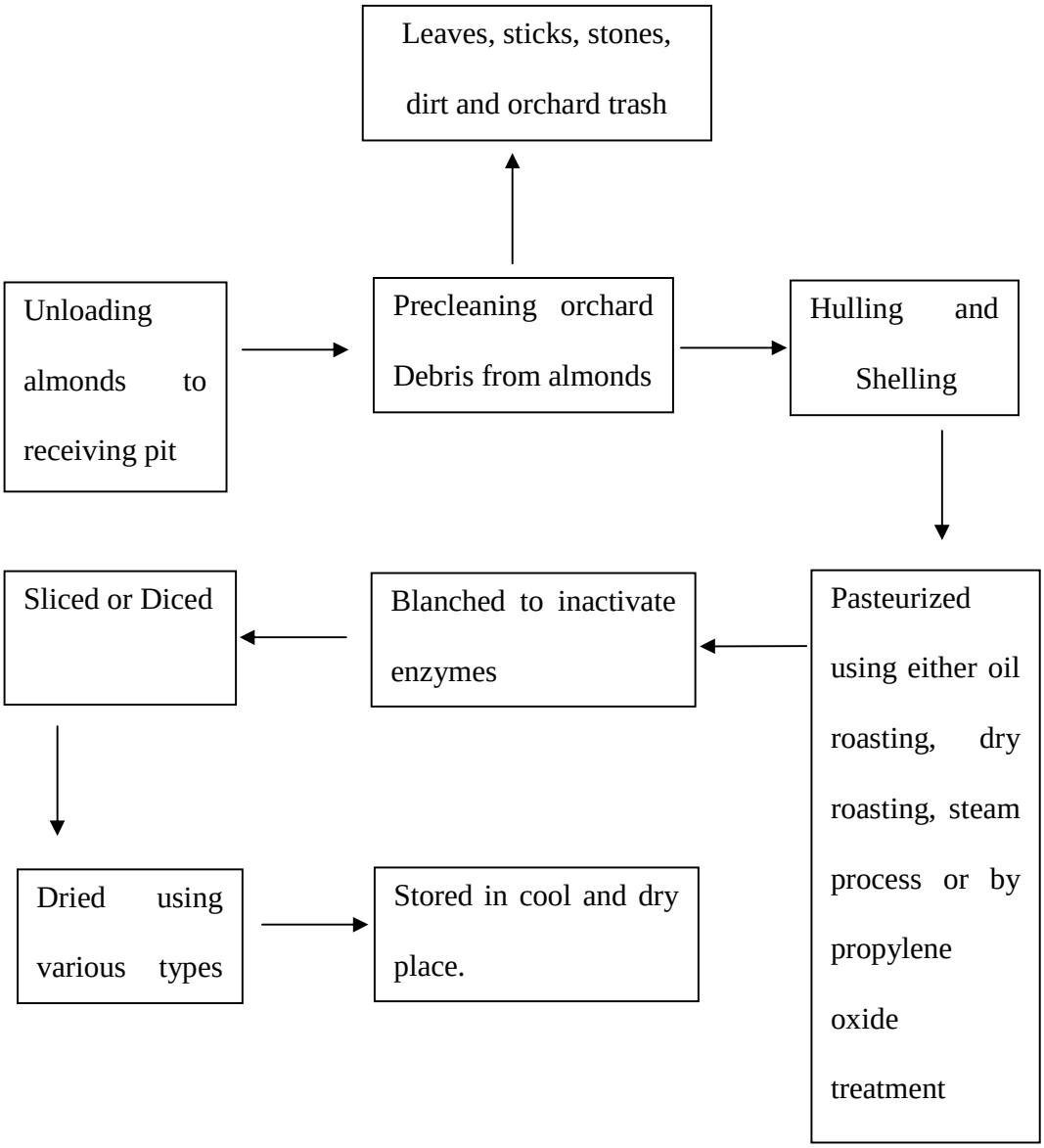

Figure 4: Schematic of almond processing

2.1.3 Pasteurization

According to United States department of agriculture under federal marketing order 7 CFR part 981 it is mandatory to pasteurize California almonds. This rule was submitted to USDA and published on March 30, 2007 in the federal register and implemented since September 1, 2007. The federal law taking into consideration Almond Board of California requires a minimum 4-log reduction of salmonella bacteria by various processes such as pasteurization. According to FDA almonds are said to be pasteurized when there is 5-log reduction of salmonella bacteria but to make it more convenient 4-log reduction treatment is considered to be pasteurized (Almond Board of California, 2010).

Pasteurization is a very important measure taken to avoid any outbreaks related to Salmonella bacteria. Several outbreaks related to Salmonella Enteritidis infections were reported by Centre for Disease Control and Prevention in the year 2001 in Canada which sickened 100 people. In the year 2003-2004 about 29 patients were found to be infected in 12 states and 1 Canadian province (Center for Disease Control and Prevention 2004). In order to avoid these outbreaks various pasteurization methods suggested by Almond Board of Californian, approved by United States Department of Agriculture were employed. According to Almond board of California it is proved that pasteurization reduces bacteria causing food borne illness (Almond Board of California, 2010).

Several methods have been approved by for pasteurization by FDA that includes oil roasting, dry roasting, steam processing and propylene oxide (PPO). Organic almonds can be pasteurized using steam pasteurization to meet USDA Organic Programs national standards. Method of irradiation is not included for almond pasteurization (Almond

Board of California 2010). All the technologies used are reviewed by Almond Board of California's Technical Expert Review Panel (TERP) (Almond Board of California, 2007). The methods are as follows:

a. Oil roasting: It is a pasteurization procedure approved by FDA to achieve 5-log reduction of salmonella bacteria. It requires a minimum oil temperature of 260^{o}F for minimum 2 minutes at the coldest point (Almond Board of California, 2007).
b. Blanching: FDA has labeled blanched almonds as pasteurized when 5-log reduction of salmonella bacteria is achieved by blanching almonds at a minimum temperature of water at 190^{o}F for at least 2 minutes (Almond Board of California, 2007).
c. Steam processing: It is a surface treatment. There are two currently used propriety system accepted by TERP namely FMC JSP-1 that used HTST (high temperature short time) method having moist heat in non pressurized environment. The second is H_2O express that uses steam at low temperature. In this method the almonds are treated in package. Steam processing does not affect sensory and nutritional characteristics of almonds (Almond Board of California, 2007).
d. Propylene oxide (PPO): It is approved for use since 1958 and re-registered with Environment Protection Agency (EPA). It's a surface treatment that ensures no change of sensory and nutritional characteristics. The operating parameters for almonds kernels propylene oxide (PPO) pasteurization are shown in Table no 2 and the operating parameter for bulk packed almonds are shown in Table no 3 (Almond Board of California, 2010).

PPO Pasteurization Operating Parameters	**Operational Level**
Initial product temperature	Not less than 86^{o}F (30^{o}C)
Temperature inside chamber at start and during sterilization	117-125^{o}F(47-51^{o}C)
Chamber vacuum before PPO injection	At least 27”Hg vacuum
PPO vaporizer temperature at point of PPO injection	140 -160^{o}F (60-71^{o}C)
Initial PPO concentration in Chamber	Not less than 0.5oz PPO/ft^{3}
Chamber vacuum at completion of inert gas injection	5-6 inch Hg vacuum
Duration of pasteurization	4 hours
Aeration cycles	Not <4 and not >14
Post ventilation	100-110^{o}F (38-43^{o}C) for 2 days or above 59^{o}F (15^{o}C) for 5 days

Table 2: Operating parameters for almonds kernels propylene oxide (PPO) pasteurization. (Almond Board of California 2008).

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Initial product temperature	Not less than 86^{o}F (30^{o}C)
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Chamber vacuum before PPO injection	At least 27”Hg vacuum
PPO vaporizer temperature	140 -160^{o}F (60-71^{o}C)
PPO concentration	Not less than 0.5oz PPO/ft^{3}
Chamber vacuum at completion of inert gas injection	5- 6 inch Hg vacuum
Duration of pasteurization	4 hours
Aeration cycles	Not <4 and not >14
Post ventilation	100-110^{o}F (38-43^{o}C) for 2 days or above 59^{o}F (15^{o}C) for 5 days.

Table 3: Operating parameter for bulk packed almonds propylene oxide (PPO) pasteurization (Almond Board of California 2008).

2.1.4 Drying

Drying is a mode of preservation as it reduces water activity to eliminate growth of microbes and avoid degradative chemical reactions. The enzyme activity and temperature are important parameters for drying (Rahman, 2007). During drying of nuts, they tend to undergo several reactions that affect the quality as they produce off flavor and off color. As the temperature increases so does the rate of lipid oxidation. High temperature can lead to degradation of antioxidants and also inactivates antioxidant enzymes by denaturation. Moreover water activity also leads to lipid oxidation. The low water activity resulted due to continuous removal of water causes acceleration of lipid oxidation since it leads to lipid hydroperoxides protective water solution layer loss (Fennema's, 2008).

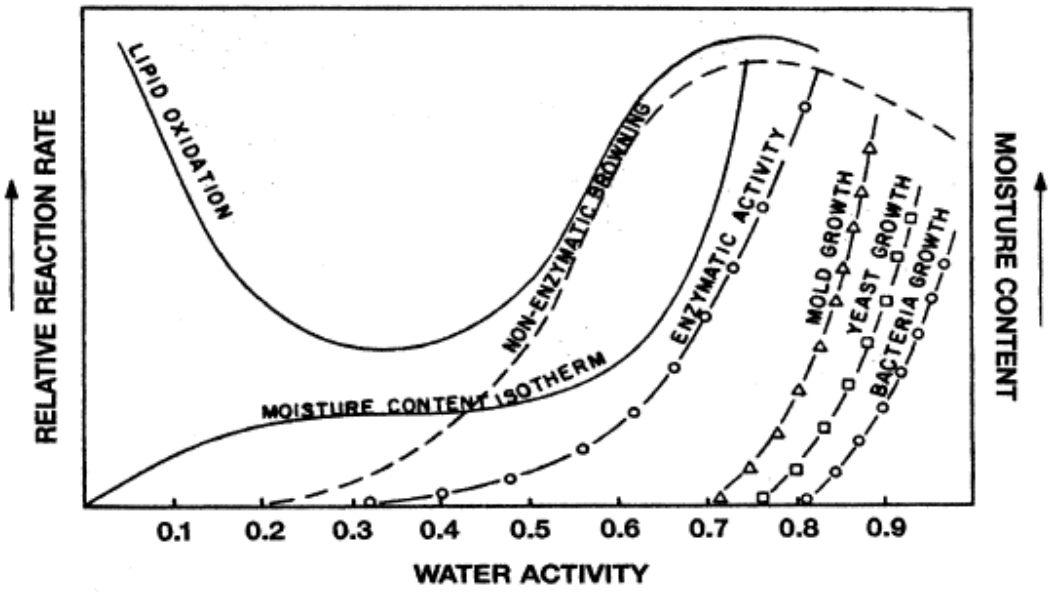


Figure 5: Food stability as a function of water activity. (Labuza, 1970)

The processing such as drying reduces water activity that results in lipid oxidation. Due to over drying free radicals are formed that causes acceleration of lipid oxidation.

The metals that act as catalyst during autoxidation has water as hydration sphere during reaction. These metals are highly active in dry state. As the water activity increases, the catalytic action of metal reduces thereby slowing the lipid oxidation. However at low water activity less water is available to mobilize polar radicals that reduce termination reaction thereby increasing lipid oxidation rate. So at low water activity the oxidation rate of lipid increases, but according to figure 5, decrease lipid oxidation was observed at water activity between 0.2- 0.4 that is a target region for optimal product quality. The lipid oxidation rate increases at a_w between 0.4-0.7. At this water activity the catalyst are mobilized that enhances breakdown of hydroperoxides to free radicals (Angelo, 1992).

So considering the fact that water activity and temperature has an effect on lipid oxidation it becomes important to select appropriate drying methods and parameters. Studies on other nuts drying are shown in literature. According to Lopez et al. 1997a indicated that rancidity reactions increases when hazelnuts are dried higher than 50^{o}C. Drying temperature of air for hazelnut should be 40-50^{o}C. A decrease in activity of enzymes peroixdase, polyphenol oxidase and lipase was observer in hazelnuts when dried between 30-70^{o}C (Lopez et al., 1997b). Therefore selecting optimum drying methods and conditions are necessary to avoid lipid oxidation. Several dryers are used for this purpose such as bin dryers, vertical continuous dryer, vertical cylindrical dryer, funnel vertical dryer and infrared dryers (Nejad et al., 2002).

Infrared drying is a method that has direct thermal application. Uniform heating is achieved by this method as compared to conventional drying since the radiation exposed material get intensely heated and temperature gradient is reduced in the material. Then after intermittent radiation is applied to this material where after heating, cooling is a

done so the moisture is displaced from core to surface and uniform heating is achieved. As compared to this, in conventional drying the moisture is removed using hot air that is circulating and conductive heat transfer takes place from core to surface result in material hardening (Hebber et al., 2001). Infrared heating has several advantages as compared to convection heating such as they help reduce quality loss by reducing heating time and heating uniformly. It also saves energy and avoids solute migration to food material (Krishnamurthy et al., 2008). In case of solid materials, it is used for thin layer drying as they have small penetration depth. However the penetration depth relays on material property and radiation wavelength.

During infrared drying of almonds they are spread into thin layer on a belt and passed beneath infrared radiators where drying occurs. The drying done at 230^{o}F showed scorching and over drying at surface and under drying center making the average of 6% moisture. Drying at 180^{o}F showed a more even drying to achieve total 6% moisture. But the product heat abuse at surface triggered oxidation of lipids due to over drying leading to oxidative rancidity and decrease in shelf life of almonds.

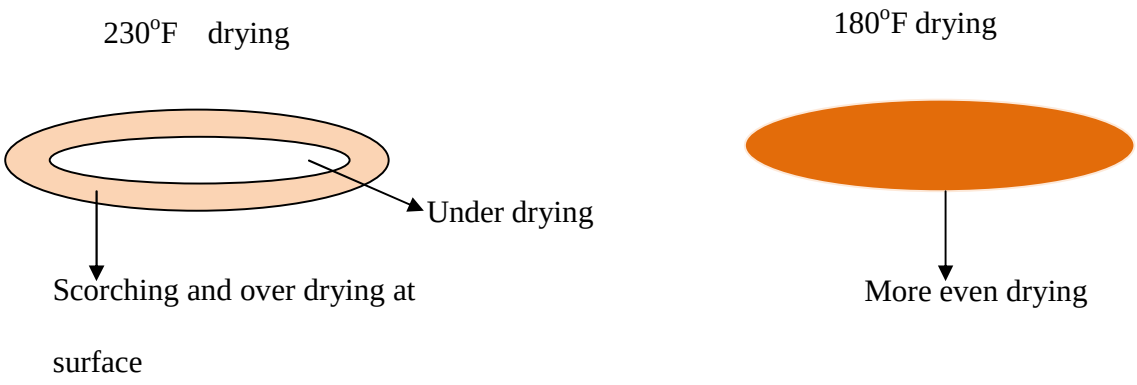


Figure 6: Showing effect of drying temperatures

2.1.5 Storage

Storage is a mode of extending the shelf life of a product. The storage condition such as moisture, temperature, insect infestation, packaging etc. are responsible for maintaining nutritional value of a product. The temperature of storage is of utmost importance as it leads to lipid oxidation. Decreasing the undesirable chemical changes of the almonds to maintain the sensory quality and components with favourable nutritional properties within the shelf life is still a challenging problem for the food industry. Therefore optimizing storage conditions is important. Optimum storage conditions for almonds are given in Table 4.

Store under cool and dry conditions (<10°C/50ºF and <65% relative humidity)
Almond moisture should be maintained at 6% or less
Avoid exposure to strong odors as almonds can absorb odors of other materials if exposed for prolonged periods
Protect from insects and pests
Roasted products must be protected from oxygen. Nitrogen flushing and/or vacuum packaging are two options
If kept under cold storage conditions (<5^{o}C/41ºF and <65% relative humidity), whole natural almonds can be stored for about two years.

Table 4: Optimum storage conditions (Almond Board of California)

2.2 Possible Alternation: Mechanism

Lipid oxidation is a major reason of food spoilage. It leads to deterioration of foods quality by oxidation of unsaturated lipids that produce off flavor and off odor. The main

chemical reactions likely to affect the lipid fraction during almonds processing and storage are hydrolysis, oxidation, isomerization of ethylenic bonds and cyclization reactions. Several factors affect lipid oxidation such as temperature, oxygen, fatty acid composition, water activity and irradiation. The consequences of these reactions on the lipids and their repercussions on human nutrition and health have been a subject of several research works in the field of lipid oxidation. Therefore, a better understanding of the various mechanisms of lipid oxidation to identify the specific changes which take place during processing and the compounds formed during the storage process are of a great importance for the improvement of stabilization methods (Ferioli, 2007).

2.2.1 Autoxidation

Autoxidation is the reaction where molecular oxygen reacts with organic compounds under mild conditions yielding hydroperoxides and other oxygenated compounds. It is mainly a free radical chain reaction described in terms of initiation, propagation and termination process. Lipid oxidation is also considered to proceed in the manner similar to organic compounds via free radical chain mechanism (Frankel, 1998).

2.2.1.1 Initiation

During initiation lipid free radicals are formed as unsaturated lipids (RH) lose a hydrogen radical ($H^{\bullet}$) in presence of initiator (I).

$$RH \xrightarrow{I} IH + R^{\bullet}$$

The initiation occurs by various mechanisms such as thermal dissociation of hydroperoxides, hydroperoxide decomposition by metal catalysis and by exposure to

light in presence of sensitizer. The initiation of lipid oxidation mechanism is still not understood completely (Frankel, 1998).

2.2.1.2 Propagation

During propagation stage lipid peroxyl radicals ($ROO^{\bullet}$) are formed when alkyl radical of unsaturated lipids ($R^{\bullet}$) having labile hydrogen reacts with molecular oxygen. This lipid peroxyl radical further reacts with unsaturated lipids (RH), forms hydroperoxides (ROOH) and lipid free radical ($R^{\bullet}$) (Frankel, 1998).

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$$

$$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$$

2.2.1.3 Termination

During termination stage lipid peroxyl radicals reacts with each other and causes self destruction by forming non-radical products (Frankel, 1998).

$$ROO\bullet + ROO\bullet \longrightarrow \text{non radical products}$$

2.2.2 Enzymatic Degradation

The free fatty acids are formed due to enzymes that cause hydrolysis of triacylglycerols or lipolysis of fats. It is found that lipoxygenases are the major enzymes that oxidize polyunsaturated fatty acids. Lipoxygenase (LOX) enzyme is specific towards oxidation of polyunsaturated fatty acid (Ferioli, 2007). Here enzyme with iron atom in its center is activated by the oxidation of iron to form oxidized iron (Fe^{3+}) which

is promoted by peroxide. This enzyme then catalyzes the hydrogen abstraction from methylene-interrupted carbon and forms alkyl radical by converting back to ferrous state. Further a peroxyl anion is formed by donating electron to peroxyl radical which reacts with hydrogen to form hydroperoxide and release fatty acid. Then after the enzyme abstracts hydrogen from fatty acid and gets converted to ferrous state when the oxygen is exhausted and yields alkyl radical (Fennema's, 2008).

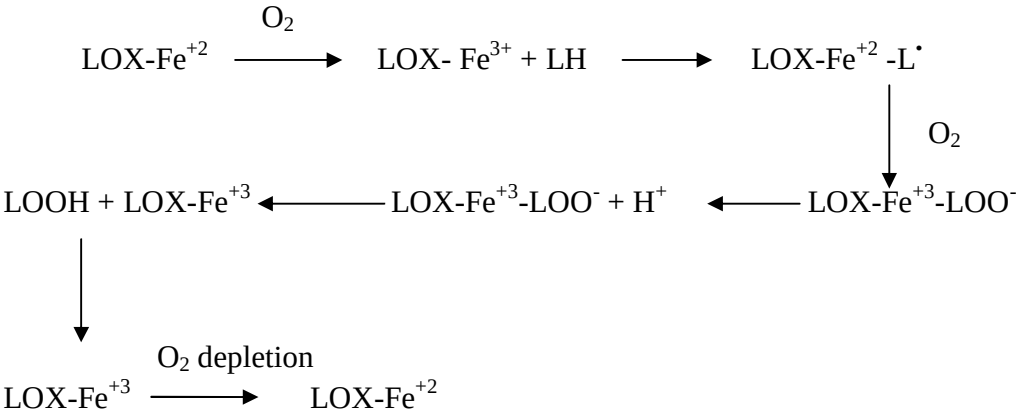


Figure 7: Mechanism of formation of hydroperoxide by lipoxygenase enzyme.

(Fennema's, 2008)

2.2.3 Hydrolysis

The hydrolysis of triacylglycerol can occur due to enzymatic action or non enzymatic action (i.e. by heat and moisture). During this free fatty acids are liberated from the glycerol back bone resulting in short chain free fatty acids and long chain free fatty acids responsible for development of off flavor and soapy flavor respectively. During hydrolysis of triacylglycerol by lipase enzyme, they act at the interface between insoluble lipids and aqueous phase to catalyze acyl group transfer to water. The processing

(temperature and moisture) and storage conditions, results in hydrolysis of triacylglycerol (Hermansyah et al., 2007)

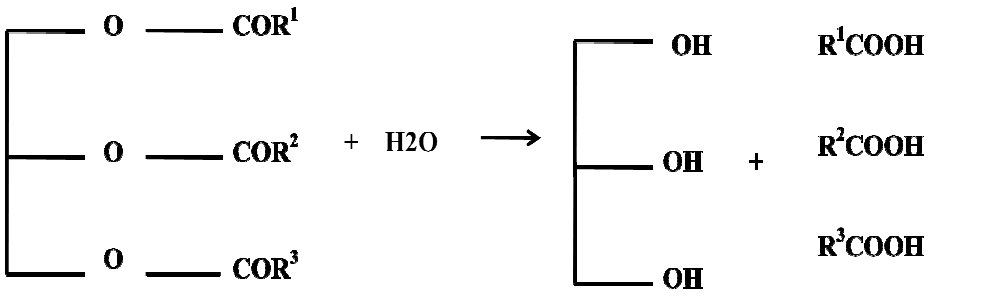


Figure 8: Formation of free fatty acid due to hydrolysis of triglycerides.

(Hermansyah et al., 2007)

2.3 Methods to Measure Lipid Oxidation

Lipids are vital component as they provide the food matrix a balanced nutritional value and pleasant flavors. However, due to their oxidative reactions the quality of food deteriorates as it leads to off flavor, off odor, change in color, loss of essential amino acids, liposoluble vitamins and natural antioxidants, etc. (Shahidi & Zhong, 2005). In order to measure the lipid oxidation in food systems several analytical methods are used depending on their application. The methods are categorized into five groups as follows:

a. Oxygen absorption: It is measured by two methods weight gain and Head space oxygen uptake. The weight gain method measures mass change that occurs due to oxygen intake during autoxidation of oil or fat. The head space oxygen uptake measures the pressure drop in oxygen (Shahidi & Zhong, 2005).

b. Initial substrate loss/ measurement of reactant change: It is measured by using Gas Chromatography (Shahidi & Zhong, 2005).
c. Free radical formation: It is measured by electron spin resonance (ESR) spectrometric assay (Shahidi & Zhong, 2005).
d. Primary oxidation products formation: It is measured by several methods. All the methods are listed below in Table 5 (Shahidi & Zhong , 2005).
e. Secondary oxidation products formation: It is measured by several methods. All the methods are listed below in Table 6 (Shahidi & Zhong, 2005).

Method	Principle	Measurement	Sensitivity	Applications
Iodometric Titration (PV)	Reduction of ROOH with KI and measurement of I_2	Titration with $Na_2S_2O_3$	≈0.5-meq/kg fat	Fats and oils
Ferric ion Complex (PV)	Reduction of ROOH with Fe^{2+} and formation of Fe^{3+}complexes	Absorption at 500-510 nm of the red complex with SCN Absorption at 560 nm of the blue complex with xylenol orange	≈0.1-meq/kg fat ≈0.5-meq/kg sample	Fats ,oils and food lipids All samples
FTIR (PV)	Reduction of ROOH with TPP	Absorption at 542 cm^{-1} of TPPO	≈0.2-meq/kg fat	Fats and oils
Chemiluminescence (PV)	Reaction with luminol in the presence of heme catalyst	Chemiluminescence emission of oxidized luminol	≈1 pmol	Fats and oils
GC-MS (PV)	Reduction of ROOH to ROH derivative	ROH derivatives	From ng to fg depending on technical details, amt of sample and detection system.	All samples
UV spectrometry (conjugated dienes and trienes)	Estimation of conjugated dienes and trienes	Absorption at 230-234 nm and 268 nm	≈0.2 meq/kg lipid	All samples

Table 5: Methods for analysis of primary oxidation product (Shahidi & Zhong, 2005).

Method	Compounds	Comments	Applications
TBA	TBARS, mainly maloaldehyde	Spectrometry technique can be carried on whole sample	All samples, especially fish oils
p- Anisidine	Aldehydes, mainly alkenals	Absorption at 350 nm standard method	Fats and oils
Carbonyls	Total carbonyls or specific carbonyl compounds formed	Spectrometry techniques and HPLC for total or specific carbonyl compounds	Fats and oils
OSI method (Racimat and oxidative stability instrument)	Volatile organic acids	Monitoring changes in conductivity rapid and automated	Fats and oils
Gas chromatography	Volatile carbonyls and hydrocarbons	Direct headspace rapid analysis	All samples

Table 6: Methods for analysis of secondary oxidation product (Shahidi & Zhong, 2005)

In the present study, the oxidation of almonds lipid fraction has been monitored using four different methods which include Gas Chromatography-Mass Spectrometry (GC-MS) for semi-volatile and volatile oxidation products, Titration method for Free Fatty Acid (FFA), UV- Spectrometry for conjugated dienes and trienes and Iodometric titration for Peroxide value (PV).

2.3.1 Iodometric Titration (Peroxide value - PV)

Peroxide value is the indicator of total content of hydroperoxides formed by the reaction between oxygen and unsaturated fatty acid and is referred as primary oxidation products. Hydroperoxides have no flavor or odor but can break down to form volatile and non volatile compounds referred as secondary oxidation products such as aldehydes and ketones, which have a strong, disagreeable flavor and odor. Generally, the hydroperoxides are stable at room temperature; however, in the presence of prooxidants such as transition metals (Fe, Cu) the alkoxy radicals are decomposed as a result of oxygen-oxygen bond homolytic cleavage. As the alkoxy radical is unstable they form carbonyl compounds having low molecular weight by undergoing homolytic β-scission of carbon-carbon bond (Akoh & Min, 2008). During the initial oxidation stage the decomposition rate of lipids is reflected by formation rate of hydroperoxides reflects. Therefore, it is necessary to measure the degree of oxidation at its initial stage (Shahidi & Zhong, 2005).

In the year 1931 Lea and 1932 Wheeler used Iodometric method based on iodine measurement produced from potassium iodide by oil peroxides. Two errors in these methods were suggested by Mehlenbacher in 1960 which were (1) iodine absorption at

unsaturated bond of fatty acid and (2) due to presence of oxygen in the titration solution iodine is liberated from potassium iodide. To overcome this error Lea at the beginning filled the sample tube with nitrogen with an assumption that chloroform evolution will avoid oxygen from reentering the tube. Later on Wheeler also made an attempt to eliminate this error by homogenizing solution to lessen oxygen effect (GRAY, 1978).

The AOAC official method 965.33 is the most commonly used method for determination of peroxide value. This method measures the amount of peroxides groups present in oil or fat. The method is based on hydroperoxides or peroxides oxidizing iodine ion. Here the known amount of fat or oil sample is added with saturated potassium iodide that reacts with hydroperoxides as a result iodine ion is liberated. This liberated iodine ion is titrated against standardized 0.01 N sodium thiosulphate solution using 1% starch as indicator. The peroxide value is then calculated in terms of milliequivalent of peroxide per kilogram of fat (meq O_2/kg of fat). It involves following chemical reaction:

$$ROOH + 2H^+ + 2KI \longrightarrow I_2 + ROH + H2O + 2K^+$$

$$I_2 + 2NaS_2O_3 \longrightarrow Na_2S_2O_6 + 2NaI$$

Figure 9: Chemical reaction for peroxide value (Shahidi & Zhong, 2005).

This method displays several disadvantages which can be summarized as follow: It is a time consuming and labor-involving procedure. The sample size is also large so there is lot of waste generation. There are changes of errors in weighing of samples, lack of sensitivity due to difficulty in determination of endpoint, improper dissolution of sample

in chloroform and acidification with acetic acid, etc. Moreover, the iodine absorption across unsaturated bonds and iodine oxidation by dissolved oxygen is also potential errors of this method (Ruiz et al., 2001).

2.3.2 Titration Method (Free Fatty Acid - FFA)

The secondary oxidation of unsaturated aldehydes produces short chain fatty acids during oxidation of lipids. The fatty acids are also produced due to cleavage of lipid hydroperoxide and also due to hydrolytic cleavage of triacylglycerol (Eldin, 2005).

Free fatty acids are determined by dissolving the sample in solvents that can be non-polar or medium polar and thereby neutralizing fatty acid by adding ethanol or methanol. Then the samples are titrated with potassium hydroxide by adding phenolphthalein as indicator. The result is interpreted in terms of acid value which is defined as mg of potassium hydroxide required for free fatty acid neutralization in 1 gram of sample. The acid value is indicative of free fatty acids formed due to both hydrolysis and autoxidation (Eldin, 2005).

2.3.3 UV Spectrometry (Conjugate Dienes and Trienes)

Conjugated dienes and trienes are indicative of formation of primary oxidation products in fats and oils. During oxidation of unsaturated lipids, the reactive oxygen species affects the double bond on carbon, weakens the C-H bond leading to hydrogen dissociation by free radical. This free radical quenches hydrogen's single electron related to the carbon at the double bond there by leaving an unpaired electron and thus becomes a free radical. The molecular rearrangement of carbon centered free radical occur forming conjugated diene (Halliwell & Gutteridge, 1985). Thus Conjugated diene and

triene are formed by the rearrangement in double bond that occurs during formation of peroxy radical or hydroperoxide from unsaturated fatty acid by shifting the normal methylene-interrupted configuration into conjugated form (Shahidi & Zhong, 2005). Figure 8 shows the conjugated system consisting of C=O and C=O double bond. Since they are indicative of the formation of primary oxidation products, it is important to measure them so as to get information on oxidative status of fats and oils. The conjugated dienes and trienes are determined by means of ultraviolet spectrophotometry.

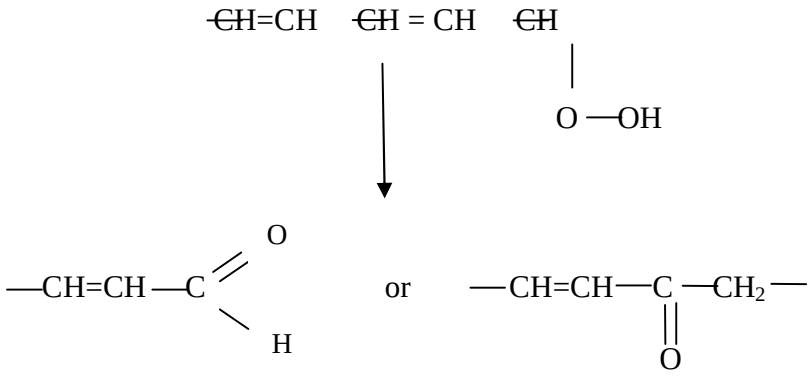


Figure 10: Formation of conjugated system containing C=C and C=O double bonds (Eldin, 2005).

It was found that an increase in ultraviolet absorption is related to oxidation of unsaturated fatty acids. In 1933 it was found that the absorption peak at 230-235 nm in ultraviolet region rises due to the formation of conjugated dienes in fats and oils. In 1934 Farmer and Sutton indicated that during early stage of oxidation absorption increases proportionately to the oxygen uptake and to the peroxide formation. In 1960 studying lipid oxidation by monitoring conjugated diene emerged as a useful technique (Shahidi & Zhong, 2005). In 1962 Privett and Blank stated that just before the end of the induction

period the plateau of UV curve occurred. Later on in 1975 Angelo et al. indicated that by studying autoxidation of peanut butter conjugated diene hydroperoxide method can be used as an index for measuring lipid oxidation as it is faster than peroxide value, doesn't involve use of chemical reagents, less time consuming and can be done with smaller samples (GRAY, 1978).

According to AOCS method Ti 1a-64 (AOCS, 1997a) the absorbance of conjugated dienes and trienes is measured at 230nm and 270nm respectively.

2.3.4 Gas Chromatography-Mass Spectrometry

As previously mentioned, during autoxidation of lipids hydroperoxides are formed. They are identified as primary oxidation products. The decomposition of these hydroperoxides yields aldehydes, alcohols, ketones, hydrocarbons, volatile organic acids and epoxy compounds known as secondary oxidation products. In order to measure the oxidative status of almonds it becomes inevitable to measure primary and secondary oxidation products. Gas Chromatography-Mass Spectrometry is an analytical method used for identification and quantification of organic compounds.

Recently MS consisting of Finnigan MAT having ion trap detector has been used as the most powerful analytical technique for lipid analysis. GC is used for separation of volatiles and MS ion trap detector is used for detection. The sample is injected into GC injection port and onto front of GC capillary column. The samples are desorbed for 5 minutes by using Cryo-Trap where the head of GC column is cooled using liquid carbon dioxide or liquid nitrogen so as to desorb the analytes. Once the samples are desorbed the Cryo- Trap heats to release the volatilized trapped analytes to the GC column for

separation. The separated analytes, in vapor phase are bombarded with electrons and forms positively charged ions. These positively charged ions are then fragmented into smaller ionized entities and propelled through a magnetic field where they are separated based on their mass to charge ratio (m/z) and collected in the sequence as the ratio increases. The ion current is amplified and displayed on the computer. The ion intensities is normalized by giving the base peak (largest peak) an arbitrary value of 100 (Christie, 1989).

3 PROBLEM STATEMENT AND OBJECTIVE

Problem Statement

Lipid fraction of almonds affects its nutritional value, flavor and stability during storage and processing as lipid is rich in unsaturated fatty acid that makes them susceptible to oxidation degradation decreasing their nutritional value and increasing economical loss. Therefore, both physical and chemical criteria are needed to monitor the oxidative status of the almond's lipid fraction.

Objectives

1. To evaluate effect of processing (drying) on oxidative status of sliced almonds during storage.
2. Compare analytical technique to evaluate oxidative status of sliced almonds during storage.

4 EXPERIMENTAL DESIGN

4.1 Materials

4.1.1 Reagents

a. Potassium iodide: Reagent plus, 99% was purchased from Sigma-Aldrich Inc. (St. Louis, MO)
b. Acetic acid, Glacial: ACS certified was purchased from Fisher Chemicals (Fairlawn, NJ)
c. Chloroform: HPLC grade was purchased from Fisher Scientific (Fairlawn, NJ)
d. Sodium thiosulfate: Volumetric standard 0.1N solution in water was purchased from Fluka analytical & Sigma-Aldrich, Co. (St. Louis, MO).
e. Starch: Corn starch 100% pure was purchased from America's choice (Montvale, NJ)
f. 95% ethanol: ACS/USP grade was purchased from Pharmco-AAPER (Brookfield, CT)
g. Diethyl ether Anhydrous: Certified ACS/ BHT stabilized was purchased from Fisher Scientific (Fairlawn, NJ)
h. Potassium hydroxide : Technical grade Flakes was purchased from Fisher Scientific (Fairlawn, NJ)
i. Cyclohexane: Certified ACS was purchased from Fisher Scientific (Fairlawn, NJ)
j. Phenolphthalein: Certified ACS was purchased from Fisher Chemicals (Fairlawn, NJ)

4.1.2 Sample Treatment

California almonds were procured for study. Almonds were pasteurized in hot water bath and blanched in chlorine water at 140^{o}F. Almonds were then sliced and dried at 180^{o}F using infrared radiation treatment and at ambient temperature. The rancid (heat abused fresh almonds) almond sample was dried at 230^{o}F that showed accelerated aging. The naturally aged sample was not processed and stored for more than 1 year at room temperature. The samples that were dried at ambient temperature and180^{o}F using infrared radiation were labeled as before and after respectively. Samples were stored at 125^{o}F for three weeks indicative of initial lipid oxidation due to accelerate storage condition.

4.1.3 Oil Sample Extraction

Almonds oil was extracted using Taby Press, type 20 a single screw extruder. It had a feed hopper where the sample was kept. The feed hopper was attached to a screw kept inside a horizontal cylinder. The screw was then attached to a press tube that was heated up to 70-100^{o}C with the help of heating collar. The screw forces nuts to pass through cylinder to obtain oil from the drilled holes and the press cake from the nozzle. 100g of almonds were weighed, passed through rotating screw to the press tube and oil was obtained in a glass beaker. Obtained oil was weighed, transferred to glass tube that was flushed with nitrogen and stored in refrigerator.

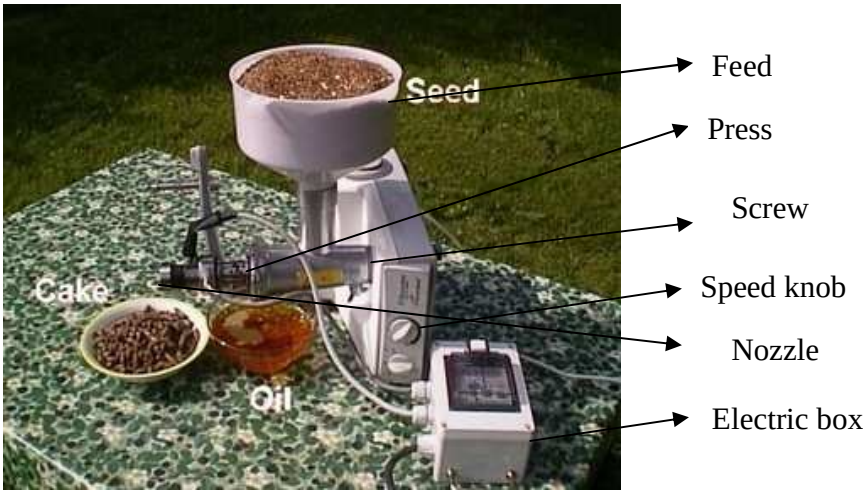


Figure 11: Showing the parts of Taby press oil press

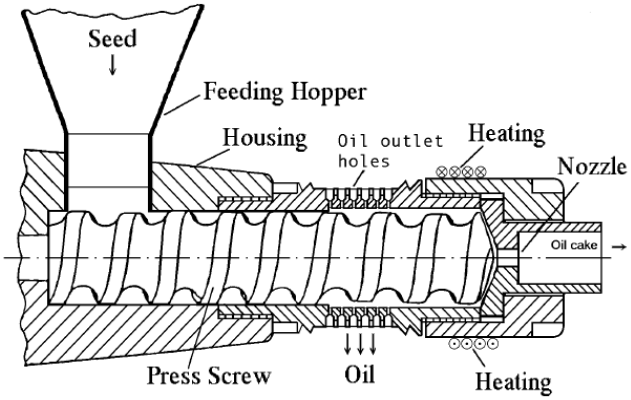


Figure 12: Schematic of single screw extruder

4.2 Methods

4.2.1 Instrumental methods

4.2.1.1 Purge and Trap

Volatile compounds for almonds were collected using short path thermal desorption system and accessories (Scientific Instrument Services inc., (SIS) Model TD-2). A GLT desorption tube packed with Tenax (4cm bed volume and 3mm inside diameter) was spiked with 10 µg of internal standard (mixture of benzene-d_6, toluene-d_8 and napthalene-d_8) for 3 minutes at 50 ml/min flow rate with nitrogen as purge gas. Trap tube spiked with internal standard was attached to glass tube containing 10gms of sample that was placed inside solid matrix sampling oven (purge and trap apparatus) for 30 minutes at 100^{o}C with nitrogen as purge gas flowing at 50 ml/min so as to trap volatile compounds. Trap tube was then after dry purged for removal of moisture with nitrogen gas flowing at 50ml/min for 30 minutes.

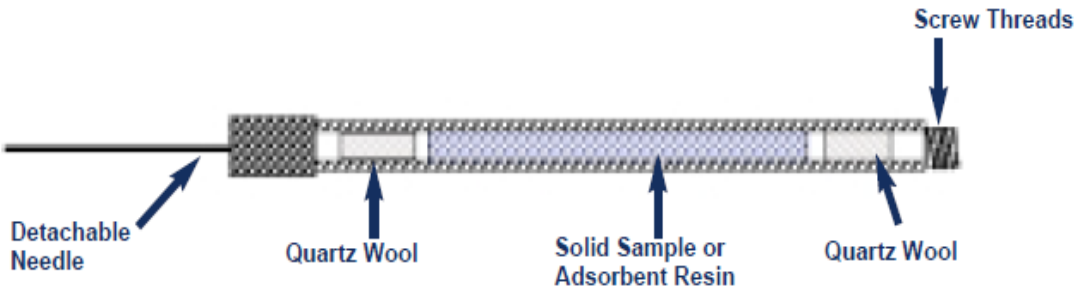


Figure 13: A GLT tenax trap tube with 3mm i.d and 4cm bed volume

(www.sisweb.com/art/pdf/td5brochure.pdf)

4.2.1.2 Thermal Desorption

A syringe was attached to volatile containing trap tube that was then attached to thermal desorber, placed above GC. Thermal desorber was heated to 250^{o}C. The purge gas used was helium having initial purge time was 10 seconds. Volatiles were injected onto GC capillary column from trap tube through thermal desorber at a temperature of 250°C having injection time as 30 seconds and desorption time as 5 minutes.

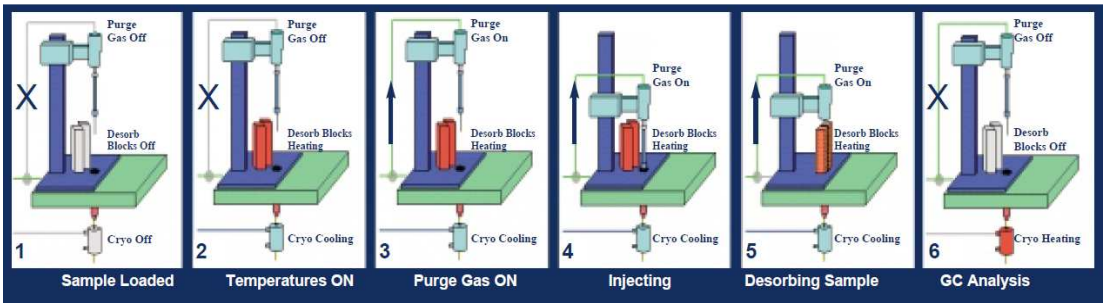


Figure 14: Schematic of working of Thermal Desorber

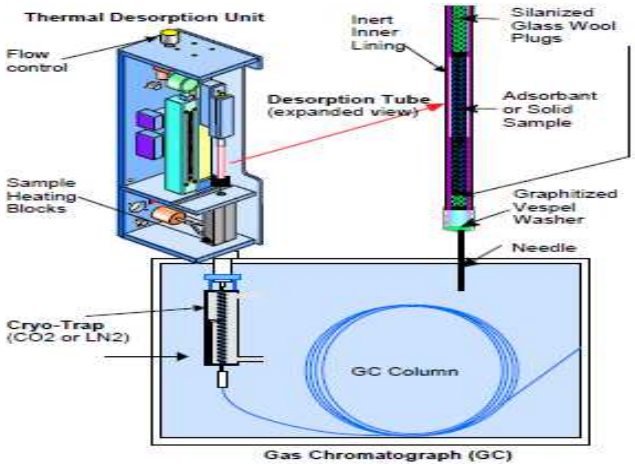


Figure 15: Schematic of injection of volatile compounds by Thermal Desorption unit

http://www.sisweb.com/art/pdf/td5brochure.pdf

4.2.1.3 Gas Chromatography and Mass Spectrometry

Almonds volatile compounds were analyzed through Varian 3400 gas chromatography model using Equity-5 capillary column (60m × 0.32mm i.d and 1.0 µm; Supelco, Bellefonte, PA) combined with Finnigan MAT 8230 MS model in electron impact EI (70eV) mode. Temperature for both ion source and injection was 250^{o}C. Volatile components were injected at 250^{o}C and allowed to desorb for 5 minutes in a split mode (100:1) onto capillary column. The oven temperature was programmed at -20^{o}C (5 minutes cryotrap) increasing temperature at 10°C/min to 280^{o}C. Capillary column was cooled using dry ice so as to have initial temperature as -20^{o}C. The carrier gas used was helium having 20psi head press. GC-MS interface line temperature was 280^{o}C. A mass range of 35-350 with scan rate at 0.6 Seconds per Decade (S/D) and inter scan time as 0.8 seconds was scanned continuously. The data was acquired with Finnigan MAT SSX software and converted using Mass Lynx software to analyze mass spectrometer data. Volatile compounds were identified by NIST mass spectral library and gas chromatographic retention times of the compounds. The concentration for each compound was measured by comparing peak area ratio with internal standard toluene-d_8.

4.2.1.4 Uv Spectrophotometry

a. Preparation of sample for Ultraviolet Spectrophotometry: Samples were prepared using spectrograde cyclohexane to a concentration of 1 mg/ml. 1 mg of almond oil was dissolved in 1ml of cyclohexane.

b. Procedure for UV Spectrophotometry: Glass cuvettes was rinsed with cyclohexane and filled with the same. The cuvettes were then placed into Uv-vis spectrophotometer (Shimadzu Uv Pharma Spec-1700) and set to auto zero for running blank. Then after the sample was placed in cuvette absorbance was measured at 230nm and 270nm. This instrument is a multicomponent analyzer having double beam with 1nm high resolution. In a double beam instrument the light passes through monochromator which is directed in one path by rotating half mirror that passes through sample at one time and reference at the next moment. These two beams are then joined by another mirror and then enter the detector where light intensities altering are automatically adjusted and readings are recorded.

4.2.2 Chemical methods

4.2.2.1 Iodometric Titration for Peroxide Value

Preparation of standards for Peroxide value

a. Preparation of Potassium iodide saturated solution (KI): It was prepared using AOAC method 965.33. Potassium iodide was prepared freshly on daily basis. Enough amount of potassium iodide was taken and saturated with freshly boiled distilled water and stored in dark place.
b. Preparation of solvent mixture of Acetic acid [CH_3COOH] and Chloroform [$CHCl_3$]: A mixture of acetic acid and chloroform was prepared using AOAC method 965.33. Acetic acid and chloroform were mixed in 3:2 proportions. A total of 2000ml was

prepared of which 1200ml of Acetic acid and 800ml of chloroform were mixed and stored in dark place.

c. Preparation of Sodium thiosulfate [$Na_2S_2O_3$]: A total of 100ml of 0.01N sodium thiosulfate was prepared as per AOAC method 965.33. 10ml of 0.1N sodium thiosulfate was dissolved in 90ml of distilled water to make a total volume of 100ml and stored in cool and dark place.

d. Preparation of 1% starch indicator: A total of 100ml of 1% starch indicator was prepared as per AOAC method 965.33. 1g of soluble starch was weighed and dissolved into cold water so as to make thin paste. 100 ml of water was boiled, mix with the thin paste of starch and boiled for 1 minute with stirring. It was stored in cool and dark place.

e. Procedure for determination of peroxide**:** Peroxide was determined as per AOAC method 965.33. The glass Erlenmeyer flask was flushed with nitrogen so as to remove as much oxygen as possible. 5 g of sample was weighed into a glass Erlenmeyer flask. 30ml of acetic acid and chloroform mixture was added to this and dissolved by constant swirling. Then after 0.5 ml of potassium iodide solution was added and kept in dark with constant swirling for about 1 min. 30ml of water as added after adding potassium iodide. 0.5 ml of 1 % starch indicator was added and titrated against 0.01N sodium thiosulfate with continuous shaking so as to release iodine from chloroform resulting into disappearance of blue color. Blank was conducted on daily basis. Peroxide value was determined using the following formula.

$$PV = \text{milliequivalent peroxide} = \frac{(S-B)*N*1000}{\text{g of fat}} \Big/ \text{Kg oil or fat}$$

Where S= ml of sodium thiosulfate, N= normality of sodium thiosulfate, B is the volume of sodium thiosulfate in blank (ml) and 1000 is the conversion unit (g/kg).

4.2.2.2 Titration Method for Free Fatty acid

Preparation of standards for Free Fatty Acid

a. Preparation of solvent mixture 95% ethanol and diethyl ether: A mixture of 95% ethanol and diethyl ether was prepared in 1:1 proportion volume/volume. A total of 2000ml was prepared of which 1000ml of 95% ethanol and 1000ml of diethyl ether were mixed and stored in dark place.
b. Preparation of 0.1M potassium hydroxide KOH: 5.61 g of potassium hydroxide was weighed and dissolved in 95% ethanol to make up a volume of 1000ml.
c. Preparation of 1% phenolphthalein: 1g of phenolphthalein was weighted and dissolved in 100 ml of 95% ethanol to make up a volume of 100ml.
d. Procedure to determine Free Fatty acid: 0.3 g of sample was weighed into a glass Erlenmeyer flask. 50ml of solvent mixture of 95% ethanol and diethyl ether was added to this and dissolved by constant swirling. Mohr pipette was used to add 5 drops of phenolphthalein indicator. It was then titrated with 0.1M potassium

hydroxide until the color was persistent to light pink for 30 seconds. The acid value was calculated as follows:

$$\text{Acid value} = \frac{56.1*N*V}{M}$$

Where V is the volume of potassium hydroxide in ml / number of ml of potassium hydroxide, N is the normality of potassium hydroxide and M is the mass of samples in grams.

4.2.3 Sensory Evaluation

Sensory evaluation for taste and odor was conducted by a panel of four untrained members. The evaluation was done using 4 points scale where the measurement of quality attribute was assessed using a scale from 1-4 in which 1 was the excellent quality, 2 was good quality, 3 was the intermediate and 4 poor quality.

5 RESULTS AND DISCUSSION

Foods which contain high concentrations of unsaturated lipids are particularly susceptible to lipid oxidation. Lipid oxidation is one of the major forms of spoilage in foods, because it leads to the formation of off-flavors and potentially toxic compounds. Lipid oxidation is an extremely complex process involving numerous reactions that give rise to a variety of chemical and physical changes in lipids.

Reactants ⟶ Primary products ⟶ Secondary products

The reaction of unsaturated fatty acid with oxygen leads to oxidative rancidity that affects the nutritional quality by producing off flavor compounds. According to USDA nutritional database 2011 almonds are rich in fat 49% containing 12% polyunsaturated fatty acid, 30.9% of monounsaturated fatty acid and 3.7% of saturated fatty acid. Of almonds total fatty acids 90 % are unsaturated, where the main components are oleic acid (60-70%) and linoleic acid (14-26%) (Zacheo et al., 2000). Since almonds are rich in unsaturated fatty acid they are highly prone to oxidation. Moreover the processing of almonds also contributed to the oxidation.

For the study 5 sets of samples were taken. The first was control sample, second was the before samples dried at ambient temperature, third was the after sample dried at 180^{o}F and fourth was the rancid (heat abused fresh almonds) almonds dried at 230^{o}F. The fifth was naturally aged sample which was roasted and salted that gave us the typical profile of almonds at the end of their shelf life. There were two batches labeled as batch 1 and batch 2 including both before and after samples

Decrease in peroxide value, conjugated dienes and trienes and concentration of total lipid oxidation products were seen for most the after samples dried at 180^{o}F as compared to before samples dried at ambient temperature. The control sample had lowest peroxides value and concentration of total lipid oxidation products as compared to after samples. The naturally aged sample showed the highest concentration of total lipid oxidation products as compared to all samples. This indicates that the drying had positive effect on the after samples in both the batches.

5.1 Peroxide Value

Peroxide value is the indicator of a continuous hydroperoxides formed during primary oxidation of lipids that may break down to a variety of non-volatiles and volatile secondary products. Change in peroxide value of almonds was observed after storing at 125^{o}F for 3 weeks. The peroxide value of control sample was 1 meq O_2/kg of oil whereas the rancid (heat abused fresh almonds) almonds dried at 230^{o}F had peroxide value of 3.2 meq O_2/kg of oil. The naturally aged sample had the PV of 1.8 meq O_2/kg of oil. The before samples for both the batches that were dried at ambient temperature and stored for 3 weeks under accelerated aging conditions showed the peroxide value 1.4 - 4 meq O_2/ kg of oil. Whereas the “after” samples for both the batches that were dried using infrared radiation treatment at 180^{o}F showed a decrease in peroxide value ranging from 1–6 meq/kg of oil as compared to before samples except for sample 1 after (Figure 16).

According to codex alimentarius the peroxide value for virgin and cold pressed fats and oils should be maximum 15 meq O_2/kg of oil. The results of this study are in accordance with the standard since no sample had peroxide value above 15 meq O_2/kg of

oil. The study results indicate that the after samples dried at 180°F stored for 3 weeks at 125°F showed a decrease in peroxide value as compared to before samples in both the batches samples indicating positive effect of drying except for 1 after sample in batch 1. This indicates that the lipid oxidation is still in its initial stages and this phase can be extended to increase shelf life of the product. Low peroxide values in after samples indicates that the temperature for drying was sufficient to increase storage stability of almonds but was not optimum to inhibit the peroxide formation. Since drying at high temperature leads to lipid oxidation it is critical to optimize drying parameters. Due to over drying there is loss of water solution protective layer of hydroperoxides, forming hydroperoxides. No correlation was found between peroxides and sensory as peroxides are indicative of primary oxidation and sensory evaluation is indicative of secondary oxidation.

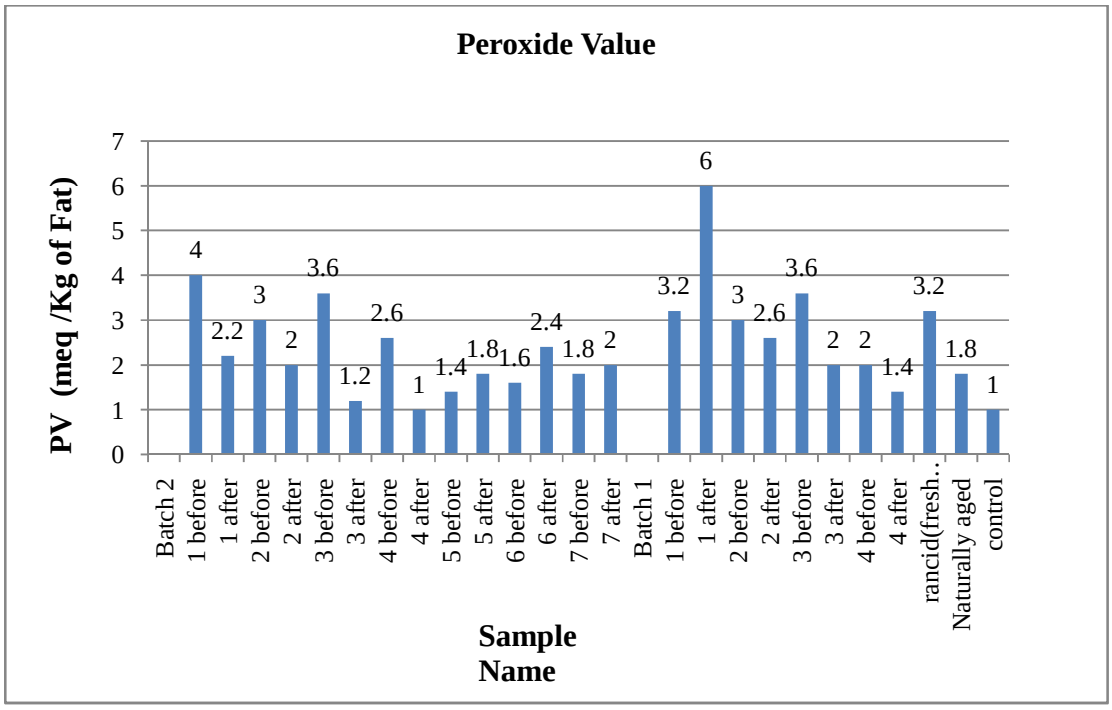


Figure 16: Concentration of Peroxide in samples

5.2 Conjugated Dienes and Trienes

Conjugated dienes and trienes are indicative of formation of primary oxidation products formed during autoxidation reaction. During the formation of hydroperoxides from unsaturated fatty acids conjugated dienes are typically produced due to the rearrangement of the double bonds. The so formed conjugated system is less stable in later stages of oxidation as they break down to form secondary oxidation products such as ketones, aldehydes and carbonyl compounds responsible for off flavor and off odor (Sanchez-Bel et al., 2005). The conjugated dienes exhibit an intense absorption at 230nm; similarly conjugated trienes absorb at 270 nm. An increase in UV absorption reflects formation of primary oxidation products formed during autoxidation reaction.

Higher absorbance was measured for "before" samples in both the batches, dried at ambient temperature ranging from 0.289nm-0.579nm as compared to "after" sample in both batches dried at 180^{o}F using infrared radiation treatment ranged from 0.218nm-0.468nm. The absorbance for control sample was 0.265nm. The rancid (heat abused fresh almonds) had an absorbance of 0.313nm and the naturally aged sample had an absorbance of 0.229nm (Figure 17). Negligible absorbance was obtained for the conjugated trienes as the lipid fraction of almonds does not contain linolenic acid. There was no absorbance for control sample, after sample dried at 180^{o}F using infrared radiation treatment and before samples dried at ambient temperature. The absorbance for rancid (heat abused fresh almonds) almonds was 0.012nm and naturally aged sample was 0.015nm (Figure 18).

Drying at high temperatures leads to lipid oxidation due to which formation of conjugated double bonds occurs resulting from rearrangement of double bond during the formation of hydroperoxides. Moreover during storage autoxidation mechanism leads to oxidative degradation of lipids forming conjugated double bonds. Therefore optimizing drying and storage conditions are of importance so as to increase shelf life of almonds. A positive effect of drying was observed since there was a decrease in absorbance for the after sample dried at 180^{o}F using infrared radiation temperature indicating that the lipid oxidation is still in its initial stages and improving drying and storage condition can delay lipid oxidation.

No correlation was found between sensory evaluation and conjugated dienes as they showed absorbance for all samples indicative of formation of hydroperoxides (primary oxidation products). These hydroperoxides formed breaks down to form secondary oxidation products, responsible for off odor and off taste detected by sensory evaluation.

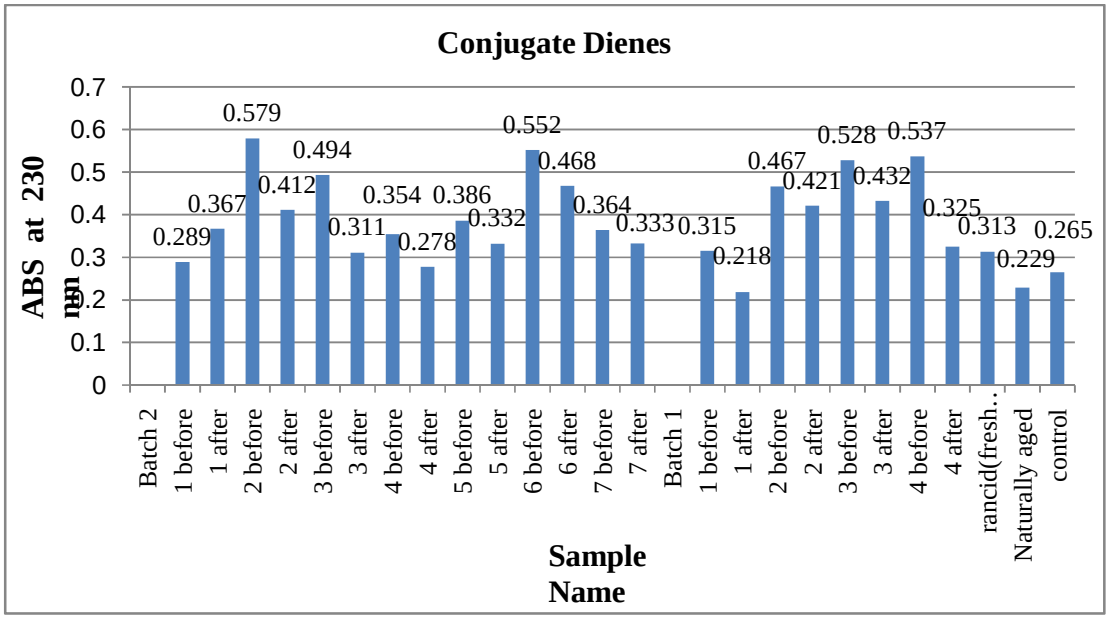


Figure 17: Concentration of conjugate dienes absorbed at 230nm

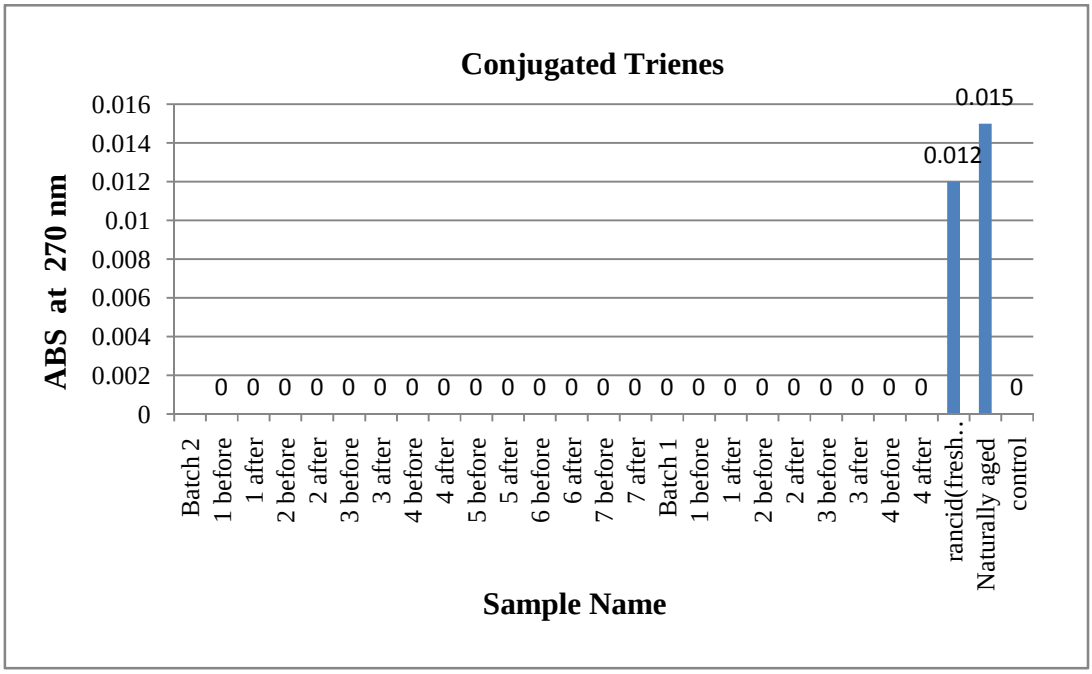


Figure 18: Concentration of conjugate trienes absorbed at 270nm

5.3 Free Fatty Acids

Free Fatty Acids are indicative of hydrolysis of triacylglycerides in presence of catalyst such as heat, moisture or enzyme. During hydrolysis the breakage of ester bonds occur forming free fatty acids and glycerol. The short chain free fatty acids formed are responsible for off-flavor and off-odor. Since drying eliminates moisture, the hydrolysis of lipids is prevented. The constant value for all the samples was obtained except naturally aged sample since the processing was different and it had already reached the end of its shelf life. This indicates that there was no hydrolysis (Figure 19) proving that the drying method used had a positive effect on storage stability of sliced almonds. No change was observed for control sample or rancid (heat abused fresh almonds) almonds.

However this method does not reflect clear status of lipid oxidation in almonds as graph with constant values is obtained indicating that no free fatty acids are formed which contradicts to results found by sensory evaluation. Since free fatty acids are responsible for off odor, no correlation of this data was found to sensory evaluation data as oxidative rancidity products were identified to be responsible for off odor and off flavor. The oxidative rancidity compounds consist of aldehydes, ketones, furans and alcohols that are also contributes greatly to off odor. The sensory data of batch 1 and control reflected no oxidation note which is indicative of good quality where as batch 2 indicated low oxidation notes showing intermediate quality.

However, no change in acid value was obtained for any of the before and after samples. High oxidative notes were observed for rancid (heat abused fresh almonds) almonds and naturally aged almonds indicating poor quality where as the acid value for rancid (heat abused fresh almonds) almonds was constant and only change in naturally aged sample was obtained.

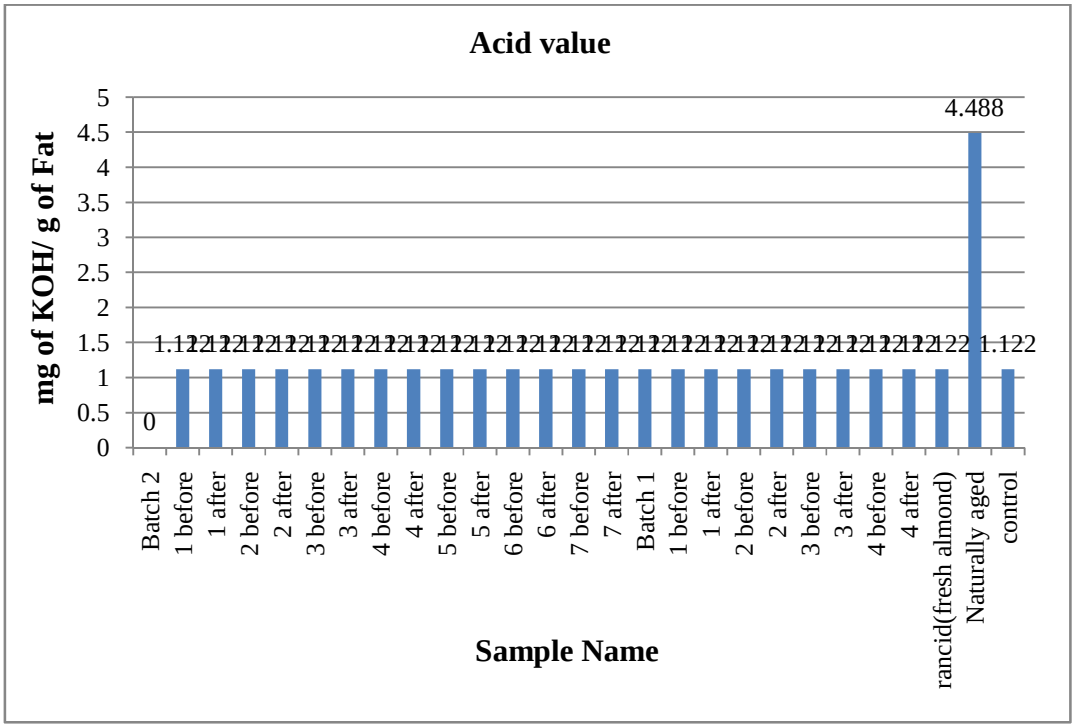


Figure 19: Concentration of free fatty acids

5.4 Gas Chromatography/Mass Spectrometry

The autoxidation of unsaturated fatty acids lead to formation of primary and secondary oxidation products. The secondary oxidation products such as aldehydes, alcohol and ketones are responsible for off-flavor and off-odor known as oxidative rancidity. These volatile and semi volatile compounds of almonds lipid oxidation during storage were detected using GC/MS. A total of 23 total lipid oxidation compounds were identified in batch 1. In batch 2, 45 total lipid oxidation compounds were identified in both before and after samples. The control sample had 26 total lipid oxidation compounds whereas 46 total lipid oxidation compounds were identified in rancid (heat abused fresh almonds) almonds. The lipid oxidation compounds were identified based on reference spectra using NIST library and the fragmentation pattern. The detected

compounds were ketones, aldehydes, aliphatic hydrocarbons, aromatic hydrocarbons and alcohols.

The concentration of total lipid oxidation compounds of after samples in both the batches was (range 1.94-16.63) lower as compared to before sample (range 3.37-17.82). The control sample had the lowest concentration of 0.93. The rancid (heat abused fresh almonds) almonds had concentration of 49.26 and the naturally aged had highest concentration of 60.27. Even though concentration of after sample was lower than before there is still a need to improve drying and storage parameters.

The volatile compounds identified are in agreement with the previous work done by Beltran et al., 2011. According to (Mexis et al., 2009 and Fullana et al., 2004) hexanal is considered as the indicator of oil quality as it is directly related to off flavor development and derived from autoxidation of linoleic acid that forms 13-hydroperoxides. The autoxidation of oleic acid derives nonanal (Fullana et al., 2004). Trans-2-nonenal is derived from linoleic acid oxidation (Lee et al., 2007). Tables 7 to 10 indicates total lipid oxidation compounds of batch1, batch 2, control samples, naturally aged and rancid (heat abused fresh almonds).

A good correlation was found between total lipid oxidation compounds and sensory evaluation. GC/MS identifies the total lipid oxidation compounds such as aldehydes, ketones etc. that are responsible for off odor and taste. The sensory evaluation of batch 1 samples indicates no rancidity for after and before samples where as batch 2 indicates low rancidity notes for both after and before, however they were still considered to be good quality or acceptable. The rancid (heat abused fresh almonds) almonds and

naturally aged samples were found to be poor quality and the control was found to be excellent quality. Since the lipid oxidation compounds contributes towards off odor and taste, a good correlation was found between sensory evaluation and lipid oxidation compounds as no rancidity and low rancidity notes were obtained by sensory evaluation for secondary oxidation products formed during autoxidation of hydroperoxides. Therefore the drying and storage conditions need to be optimized to extend shelf life of the product as secondary oxidation products were found in samples.

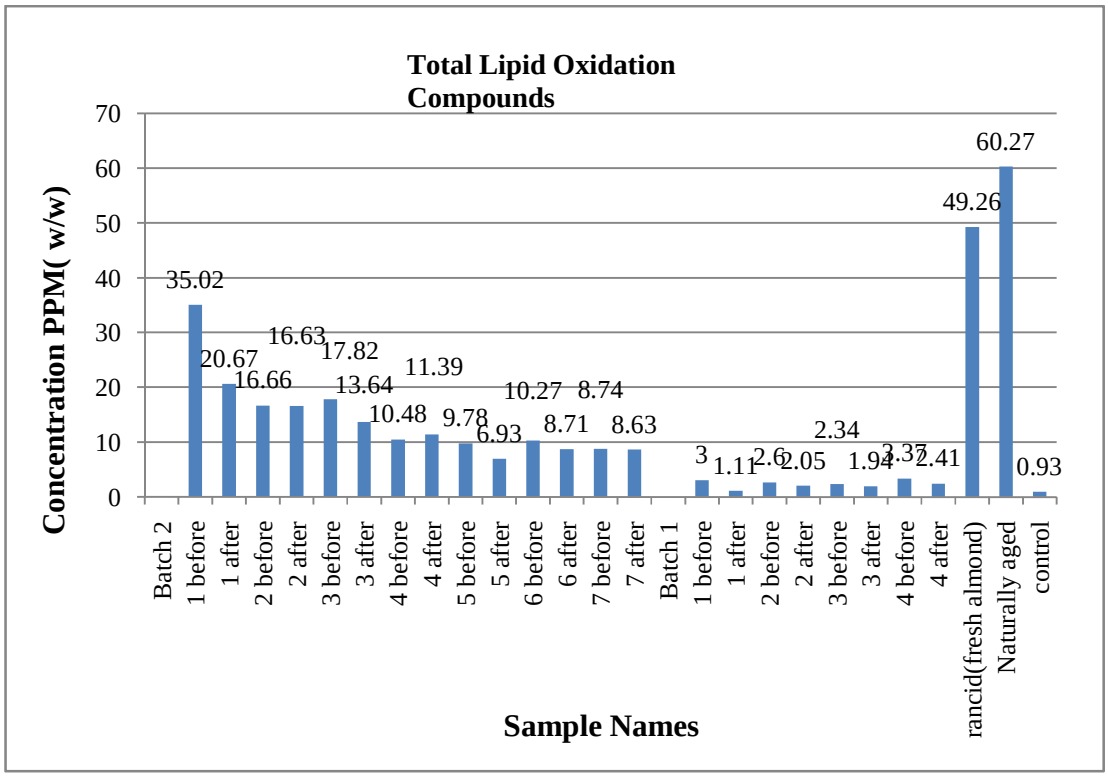


Figure 20: Concentration of total lipid oxidation compounds

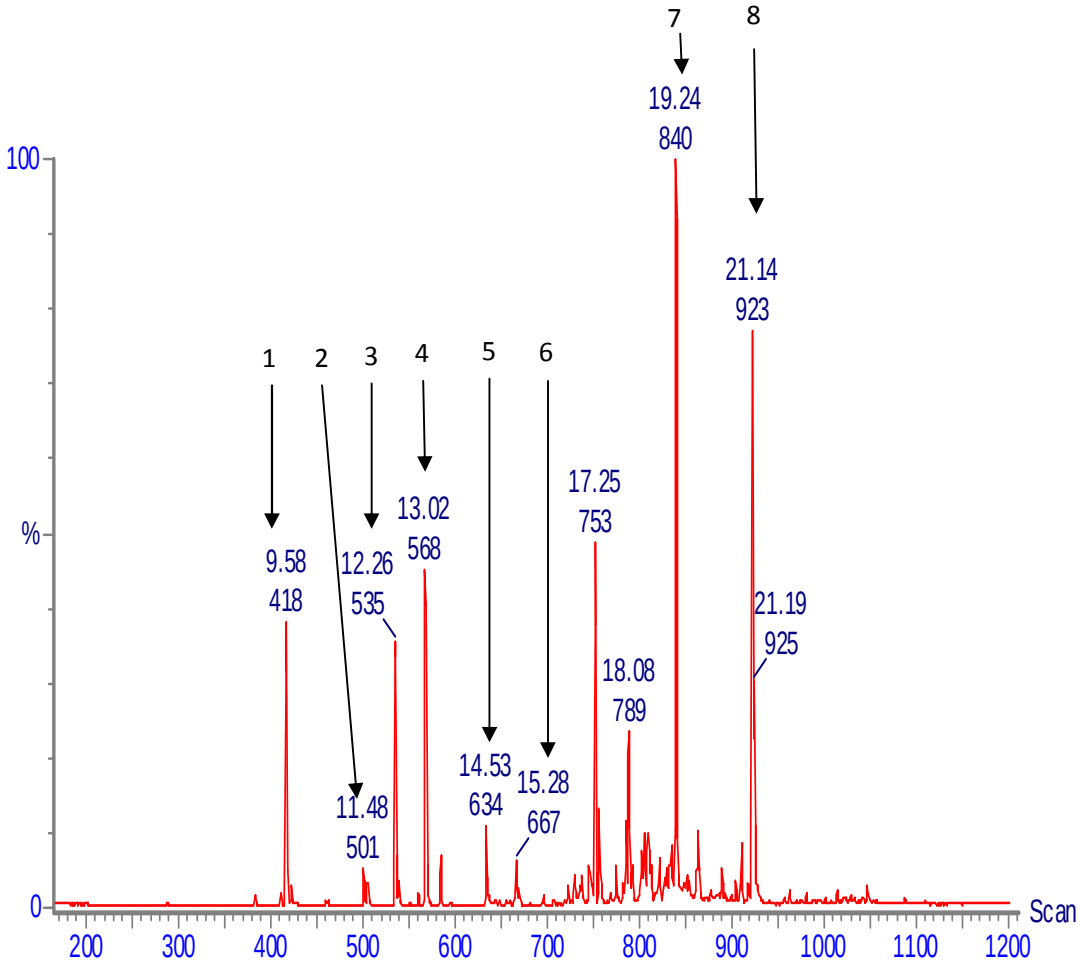


Figure 21: Chromatogram showing lipid oxidation indicator compounds in batch 1.

(1) d-8 benzene (2) isopentyl alcohol (3) d-8 toluene (4) hexanal (5) Hexyl Alcohol (6) Heptanal (7) Nonanal (8) d-8 naphthalene

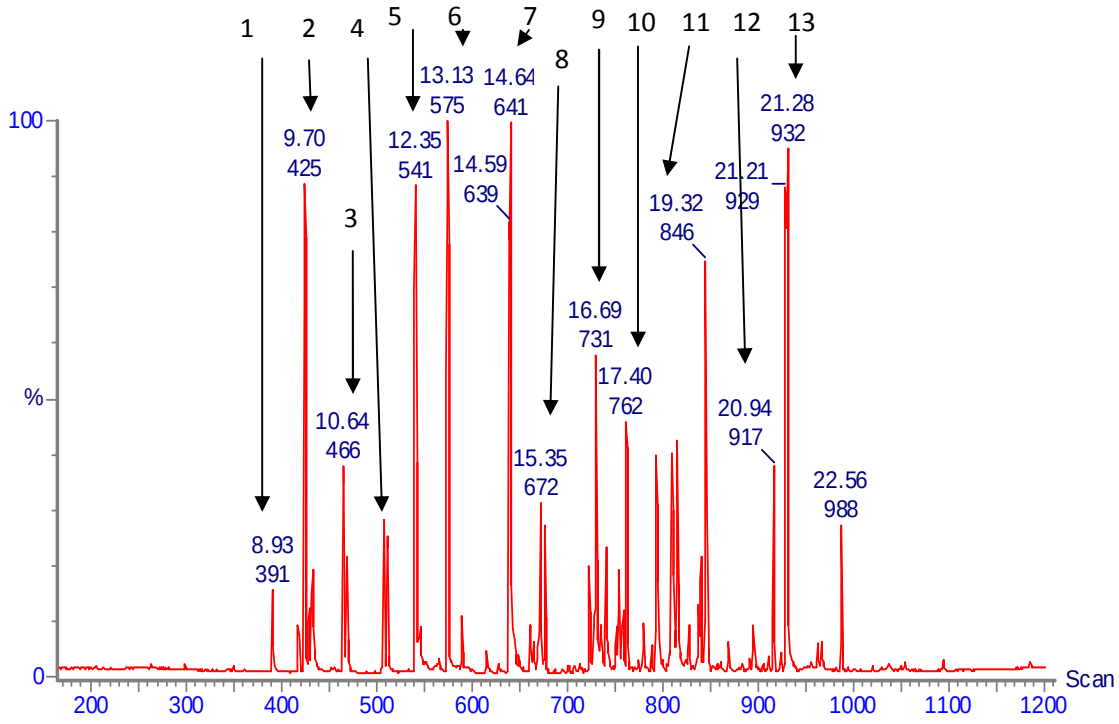


Figure 22: Chromatogram showing lipid oxidation indicator compounds in batch 2.

(1) isobutyl alcohol (2) d-6 benzene (3) pentanal (4) isopentyl alcohol (5) pentyl alcohol (6) hexanal (7) hexyl alcohol (8) heptanal (9) heptyl alcohol (10) octanal (11) nonanal (12) decanal (13) d-8 naphthalene

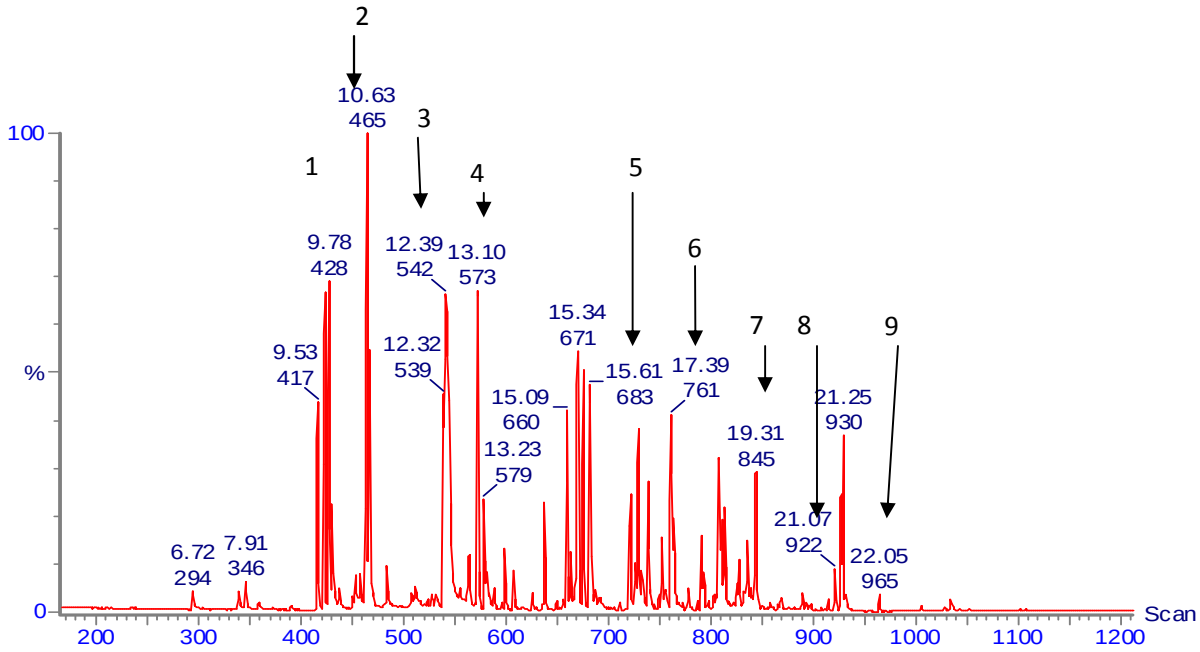


Figure 23: Chromatogram showing total lipid oxidation indicator compounds in naturally aged almonds.

(1) d-6 benzene (2) pentanal (3) pentyl alcohol (4) hexanal (5) trans-2-heptenal (6) 2-pentylfuran (7) nonanal (8) cis-2-nonenal (9) trans-2-decenal

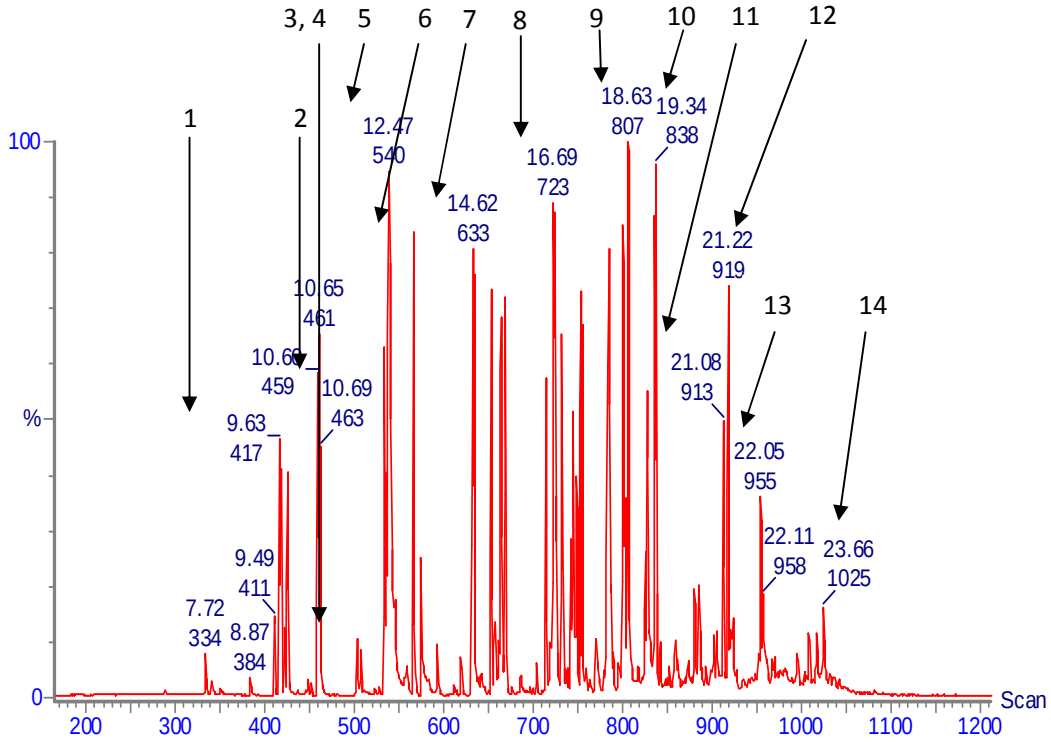


Figure 24: Chromatogram showing total lipid oxidation indicator compounds in rancid (heat abused fresh almonds) almonds.

(1)d-6 benzene (2) pentanal (3) 3-methyl-3buten-1-ol (4) isopentyl alcohol (5) pentyl alcohol (6) hexanal (7) hexyl alcohol (8) heptyl alcohol (9) octyl alcohol (10) nonanal (11) decanal (12) d-8 naphthalene (13) trans-2-decenal (14) trans-2-undecanal

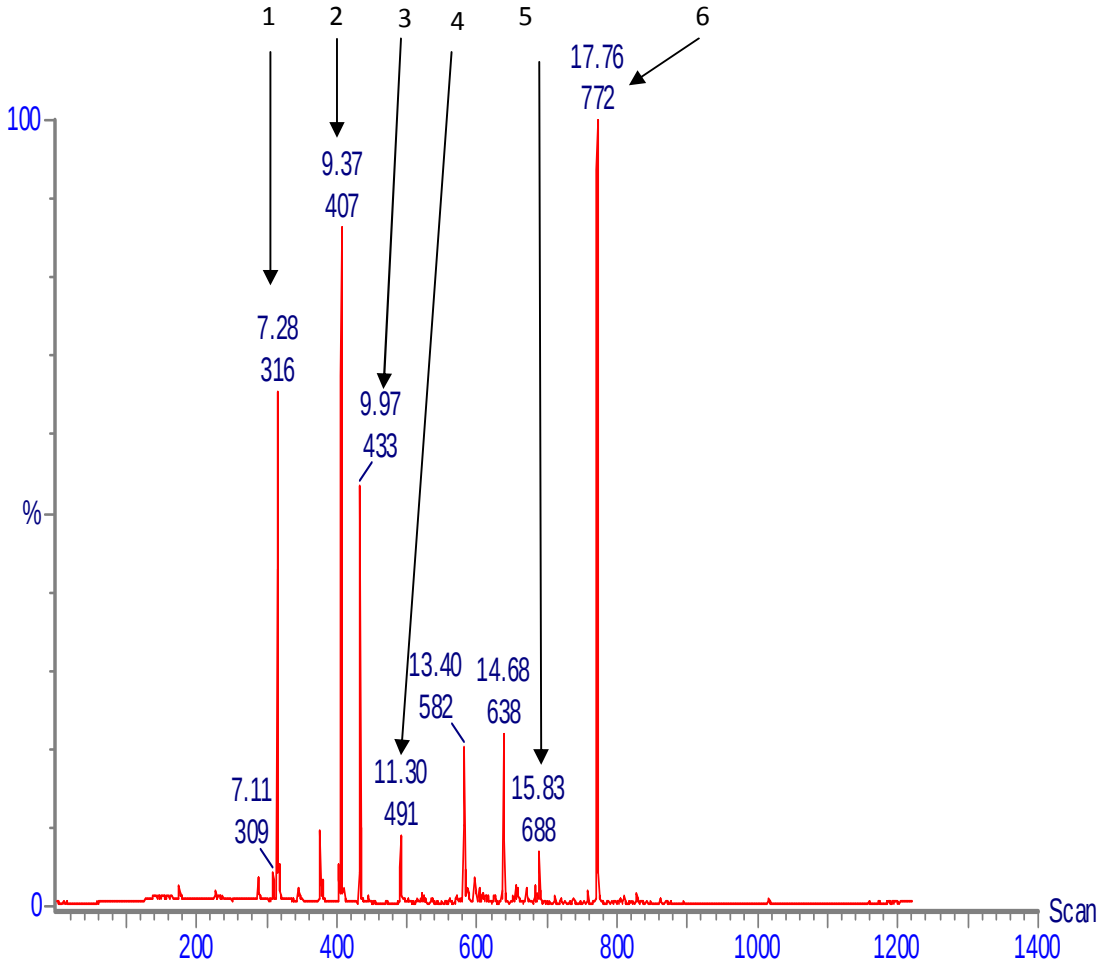


Figure 25: Chromatogram showing total lipid oxidation indicator compounds in control sample.

(1)d-6 benzene (2) d-8 toluene (3) hexanal (4) hexyl alcohol (5) nonanal (6) d-8 naphthalene

Samples	Conc. of Hexanal PPM(w/w)	Conc. of Total Lipid Oxidation Indicator PPM(w/w)
Batch 2		
1 before	2.649	35.02
1 after	1.5	20.67
2 before	2.077	16.66
2 after	3.873	16.63
3 before	2.204	17.82
3 after	2.257	13.64
4 before	1.157	10.48
4 after	1.647	11.39
5 before	1.7	9.78
5 after	1.216	6.93
6 before	1.517	10.27
6 after	1.372	8.71
7 before	1.723	8.74
7 after	1.694	8.63
Batch 1		
1 before	0.716	3
1 after	0.348	1.11
2 before	0.583	2.6
2 after	0.393	2.05
3 before	0.398	2.34
3 after	0.385	1.94
4 before	0.587	3.37
4 after	0.641	2.41
rancid(fresh almond)	2.688	49.26
naturally aged	4.736	60.27
control	0.49	0.93

Table 7: Total lipid oxidation indicator compounds and hexanal concentration in all the samples.

Peak No	LOIC	Concentration PPM (w/w)
1	butanal	0.009
2	2-butanone	0.004
3	2-butanol	0.004
4	isobutyl alcohol	0.93
5	1-butanol	0.165
6	propyl oxirane	0.007
7	2-pentanone	0.005
8	pentanal	0.193
9	isopentyl alcohol	0.457
10	trans-2-pentenal	0.007
11	pentyl alcohol	1.255
12	2-propylfuran+ 2-hexanone	0.028
13	hexanal	1.723
14	cis-3-hexenol	0.005
15	trans-2-hexenal	0.035
16	hexyl alcohol	1.301
17	2-heptanone	0.06
18	butyl acrylate+ 2-butylfuran	0.043
19	heptanal	0.184
20	pentyl oxirane	0.146
21	2-heptanol	0.006
22	2,4-hexadienal	0.013
23	trans-2-heptenal	0.128
24	heptyl alcohol	0.45
25	hexyl oxirane	0.157
26	3-octanone	0.019
27	2-octanone	0.055
28	2-pentylfuran	0.185
29	octanal	0.34
30	2,4,heptadienal	0.017
31	trans-2-octenal	0.355
32	octyl alcohol	0.277
33	heptyl oxirane	0.242
34	nonanal	0.46
35	limonene oxide	0.076
36	trans-2-nonenal	0.013
37	nonyl alcohol	0.077
38	decanal	0.022
39	2,4-nonadienal	0.03
40	trans-2-decenal	0.038

Peak No	LOIC	Concentration PPM (w/w)
41	undecanal	0.007
42	2,4-decadienal	0.008
43	cis-2-undecenal	0.007
44	trans-2-undecenal	0.02
45	dodecanal	0.003

Table 8: Total lipid oxidation products identified in sample 7 before of batch 2 stored for 3 weeks at 125^{o}F. Note: LOIC (lipid oxidation indicator compounds) and these compounds are found in rest of all samples of batch 2 having different concentration of each compound.

Peak No.	LOIC	Concentration PPM(w/w)
1	butanal	0.005
2	2-Butanone	0.001
3	isobutyl alcohol	0.021
4	pentanal	0.002
5	3-methyl-3-buten-1-ol	0.006
6	isopentyl alcohol	0.123
7	2-methyl-2-buten-1-ol	0.006
8	hexanal	0.587
9	trans -2- hexenal	0.001
10	hexyl Alcohol	0.145
11	heptanal	0.11
12	trans-2-Heptenal	0.001
13	heptyl alcohol	0.037
14	2-pentylfuran	0.107
15	octanal	0.14
16	trans-2- octenal	0.145
17	octyl alcohol	0.245
18	nonanal	1.528
19	nonyl alcohol	0.016
20	trans-2-nonenal	0.086
21	decanal	0.038
22	undecanal	0.014
23	dodecanal	0.01

Table 9: Total lipid oxidation products identified in sample 4 before of batch 1 stored for 3 weeks at 125^{o}F. Note: LOIC (lipid oxidation indicator compounds) and these compounds are found in rest of all samples of batch 1 having different concentration of each compound.

Peak No	LOIC	Concentration PPM(w/w)
1	butanal	0.017
2	2-butanone	0.062
3	2-butanol	0.027
4	2-methylfuran	0.002
5	1-butanol	0.92
6	2,3-pentadione	0.011
7	2-pentanone	0.054
8	pentanal	1.69
9	2-pentanol	0.005
10	2-ethylfuran	0.004
11	3-methyl-3buten-1-ol	0.008
12	isopentyl alcohol	0.266
13	trans-2-pentenal	0.017
14	pentyl alcohol	4.771
15	2-hexanone	0.173
16	hexanal	2.688
17	trans-2-hexenal	0.48
18	hexyl alcohol	2.172
19	2-heptanone	1.017
20	2-butylfuran	0.185
21	2-heptanol	0.249
22	heptanal	1.168
23	pentyl oxirane	0.981
24	trans-2-heptenal	0.689
25	heptyl alcohol	3.058
26	hexanoic acid	3.528
27	2-octanone	1.107
28	2-pentylfuran	1.176
29	octanal	6.366
30	hexyl oxirane	1.688
31	trans-2-octenal	2.098
32	octyl alcohol	2.004
33	2-nonanone	1.188
34	heptyl oxirane	1.411
35	nonanal	2.041
36	nonyl alcohol	0.276
37	cis-2-nonenal	0.083
38	trans-2-nonenal	0.34
39	2-decanone	0.178
40	decanal	0.605

Peak No	LOIC	Concentration PPM(w/w)
41	2-hexylfuran	0.394
42	trans-2-decenal	0.064
43	undecanal	0.116
44	2,4-decadienal	0.37
45	trans-2-undecanal	0.073
46	dodecanal	0.04

Table 10: Total lipid oxidation products identified in rancid (heat abused fresh almond) almonds stored for 3 weeks at 230^{o}F. These compounds were also identified in naturally aged samples having different concentration of each compound. Note: LOIC (lipid oxidation indicator compounds).

Peak No	LOIP	Concentration PPM (w/w)
1	butanal	0.01
2	2-butanone	0
3	2-butanol	0
4	isobutyl alcohol	0.03
5	pentanal	0.03
6	3-methyl-3-buten-1-ol	0
7	isopentyl alcohol	0.15
8	pentyl alcohol	0.06
9	2-methyl-2-buten-1-ol	0
10	hexanal	0.49
11	trans-2-hexenal	0.01
12	hexyl alcohol	0.17
13	2-heptanone	0
14	heptanal	0.04
15	trans-2-heptenal	0.01
16	heptyl alcohol	0.01
17	2-pentylfuran	0.07
18	octanal	0.02
19	trans-2-octenal	0.04
20	octyl alcohol	0.04
21	nonanal	0.15
22	nonyl alcohol	0.02
23	trans-2-nonenal	0.01
24	decanal	0.02
25	undecanal	0.01
26	dodecanal	0

Table 11: Total lipid oxidation products identified in control almonds stored for 3 weeks at 125^{o}F. Note: LOIC (lipid oxidation indicator compounds).

5.5 Correlation of Peroxide value and Conjugated Dienes

Peroxides and conjugated dienes are indicative of primary oxidation products during storage. A linear correlation was found between peroxide value and conjugated dienes showing the presence of primary oxidation products (Figure 26). These two methods confirm the presence of primary oxidation products which are formed as function of storage time. No correlation was obtained for peroxide, conjugated dienes and free fatty acids. Since the free fatty acid showed a constant graph indicating that there was no hydrolysis of triacylglycerol. The good correlation found between peroxides value and conjugated dienes confirm the statement from the previously published report (Shahidi et al., 1994, Wanasundara et al., 1995).

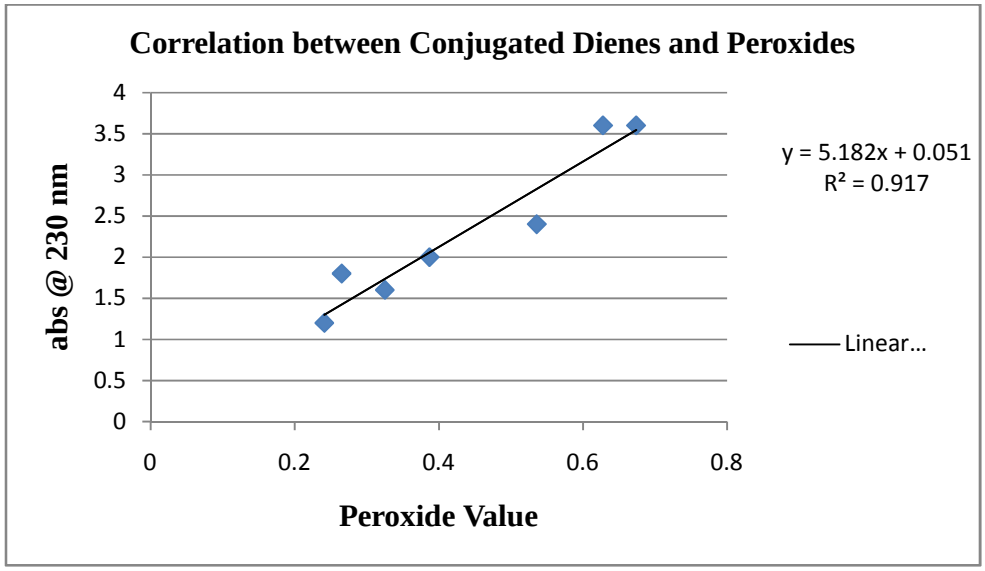


Figure 26: Correlation between Conjugated Dienes and Peroxides.

5.6 Sensory Evaluation

The organoleptic properties of almonds were studied by evaluating the sensory attribute "rancidity". The samples dried at ambient temperature had a score of 3 indicative of intermediate quality and samples dried at 180^{o}F using infrared radiation had a score of 2 indicated good quality almonds. The control sample had a score of 1 showing excellent quality where as naturally aged and rancid (heat abused fresh almonds) showed a score of 4 representing poor quality. This indicated positive effect of drying since the quality of all the samples were considered to be acceptable except naturally aged and rancid (heat abused fresh almonds) almonds.

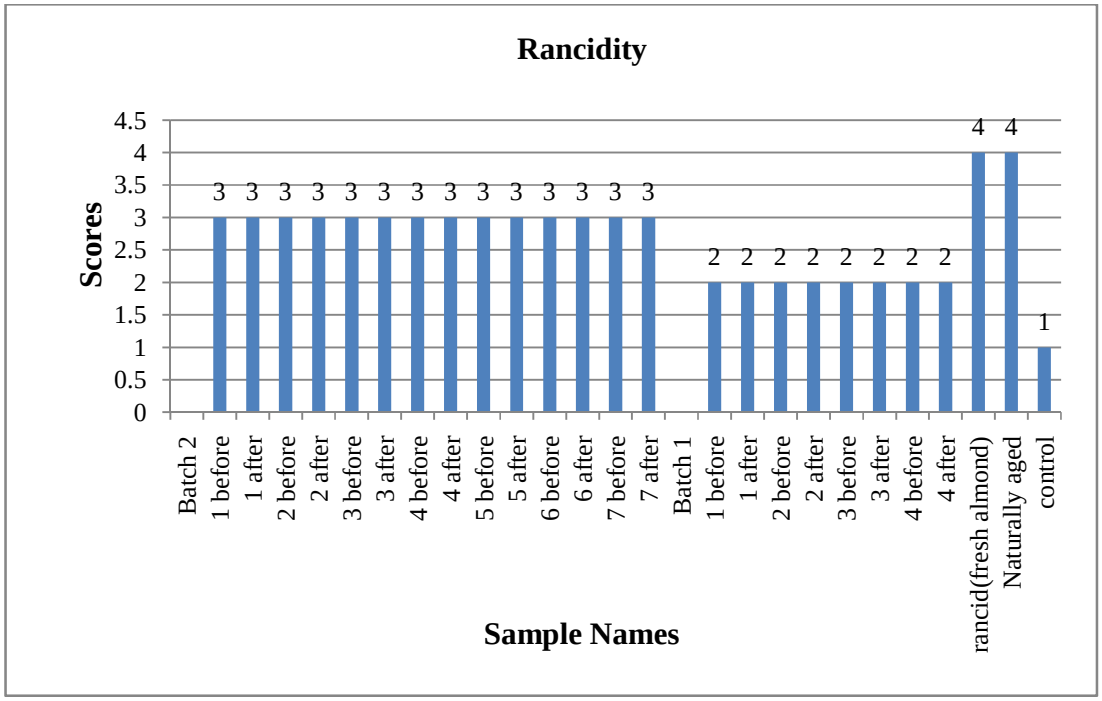


Figure 27: Showing rancidity scores of each sample

6 CONCLUSION

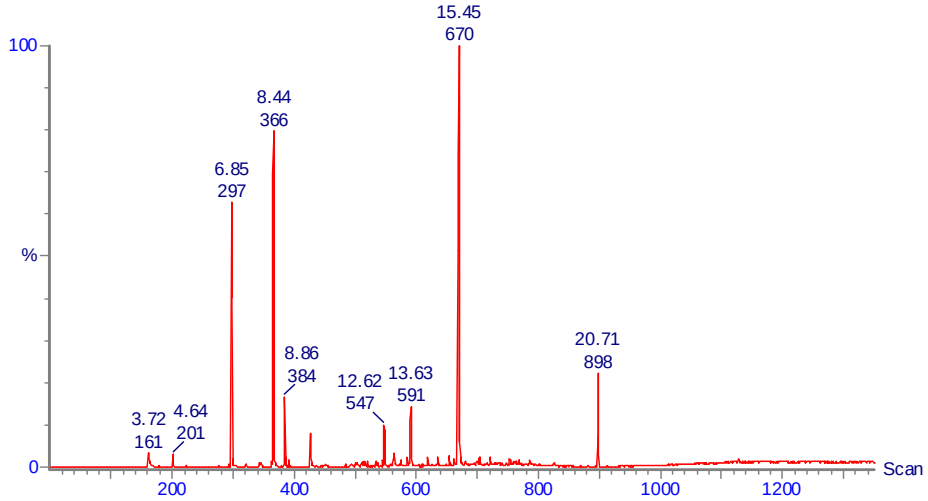
The results of this study confirm a positive impact of infrared drying on oxidative status of sliced almonds stored for 3 weeks (accelerated aging). Drying process was efficient as the content of free fatty acids remains constant during the entire storage time for all the samples except the naturally aged sample. The Peroxide value and conjugated dienes for after samples showed a decrease as compared to the ambient dried sample and rancid (heat abused fresh almonds) almonds sample. The chromatograms and mass spectra indicated presence of secondary oxidation products such as aldehydes, ketones, alcohols, aromatic compounds and aliphatic compounds in low concentration for after samples as compared to before sample indicating that the lipid oxidation is still in its primary stage. However the concentration of secondary oxidation products was lowest in control sample which indicates that the drying and storage conditions needs to be optimized.

GC/MS was found to be the most accurate analytical method to find the effect of drying on storage stability since it identifies secondary oxidation products responsible for off odor and taste. It also gives the accurate concentration of each compound formed during storage, informing about lipid oxidation status as the concentrations found for all samples were low indicating that lipid oxidation is still in its primary stage. Peroxide value and conjugate dienes indicated the presence of primary oxidation products, however since the peroxides are highly unstable they tend to react and form secondary oxidation products. The hydroperoxides formed showed a lower peroxide value and lower absorbance for conjugated dienes and trienes that did not reflect a clear picture on primary oxidative status as secondary oxidation products were identified by GC/MS and

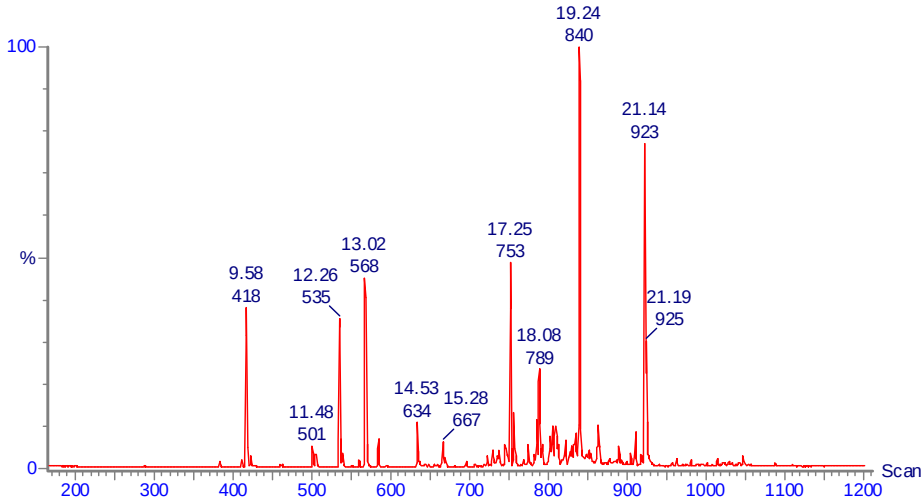
sensory evaluation. FFA formed imparts off odor and taste to almonds. The acid value measured does not reflect clear picture of oxidative status as the constant values were obtained except for naturally aged samples that was stored for more than 1 year. Since low rancidity was obtained for samples due to formation of free fatty acids or secondary oxidation products, this method did not show any formation of free fatty acids responsible for hydrolytic rancidity. Though the GC/MS is the best identified method for evaluating effect of drying on oxidative status of sliced almonds, it is advisable to combine this method with other methods which show the status of the primary oxidation. The knowledge of the content of both primary and secondary oxidation products helps to determine the rate of their decomposition and their formation, and this give a real picture on the rate of the reaction of degradation that occurs.

Further studies can be done on improving drying and storage conditions to enhance storage stability of sliced almonds. Moreover studying interaction between lipids and other compounds and their effects on lipid stability during storage could help improve drying and storage parameters.

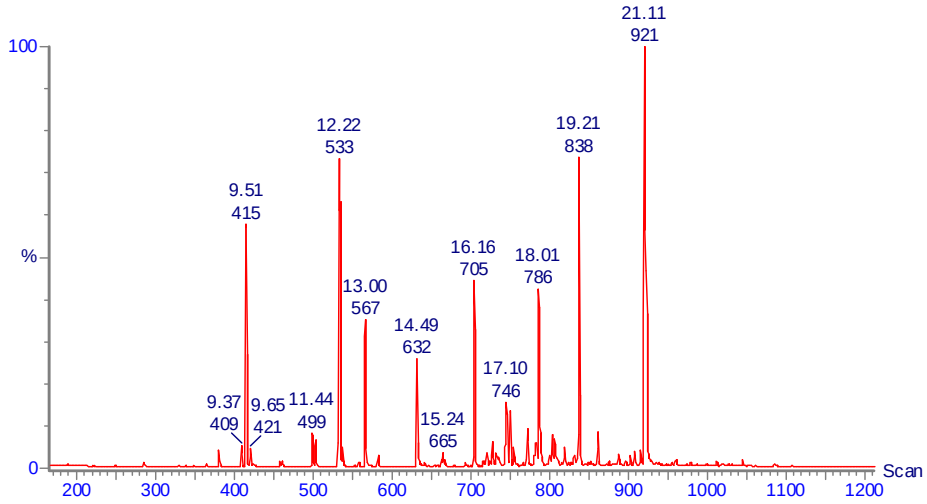
7 APPENDIX - CHROMATOGRAMS



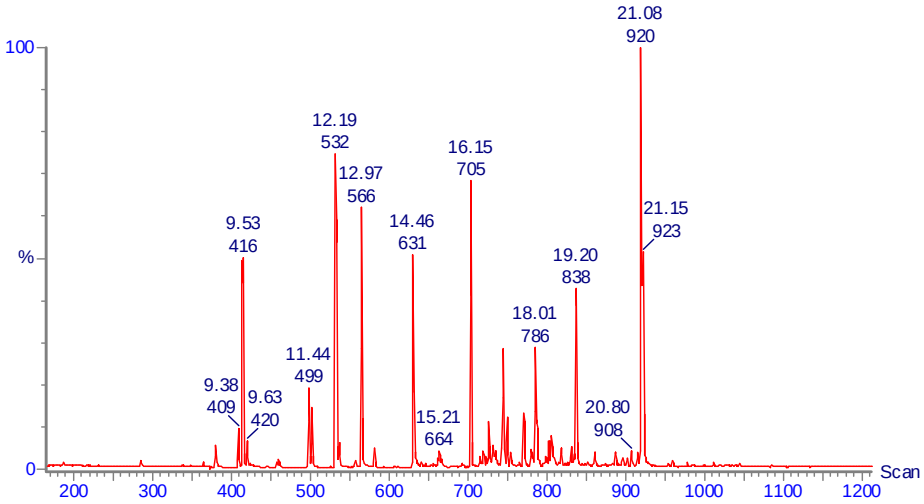
Chromatogram of control sample



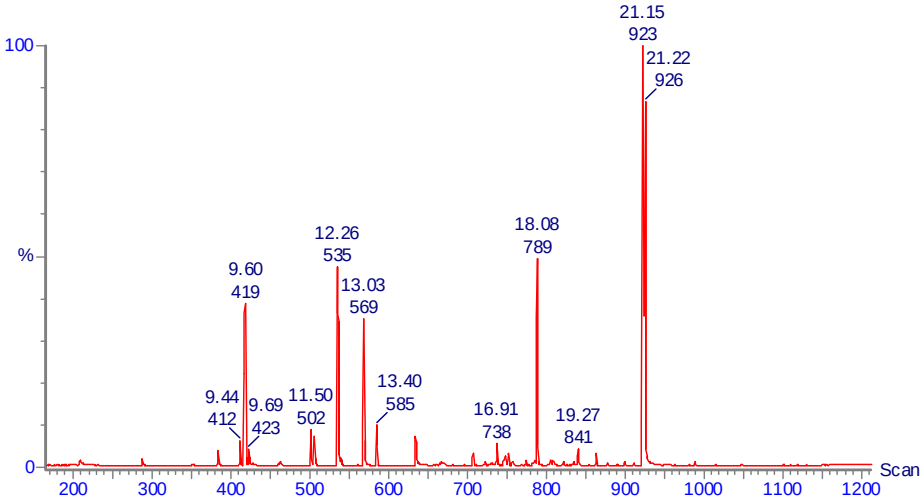
Chromatogram of sample 4 before in batch 1



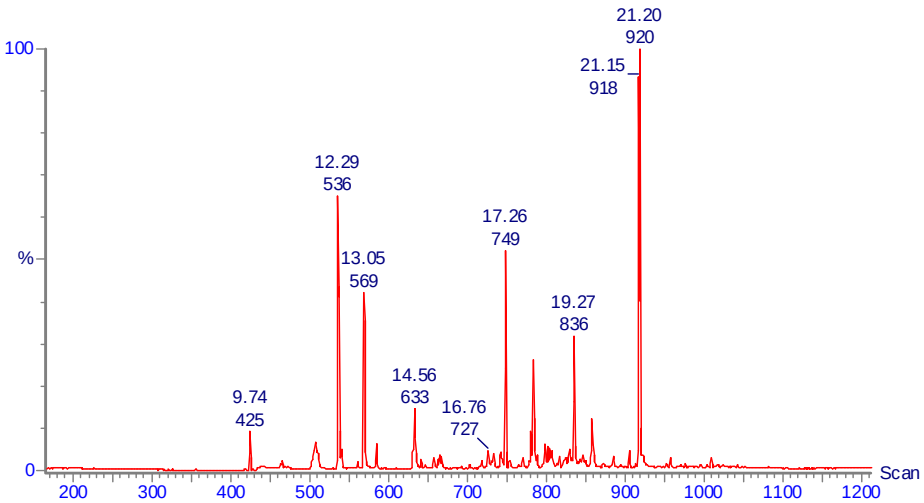
Chromatogram of sample 2 before in batch 1



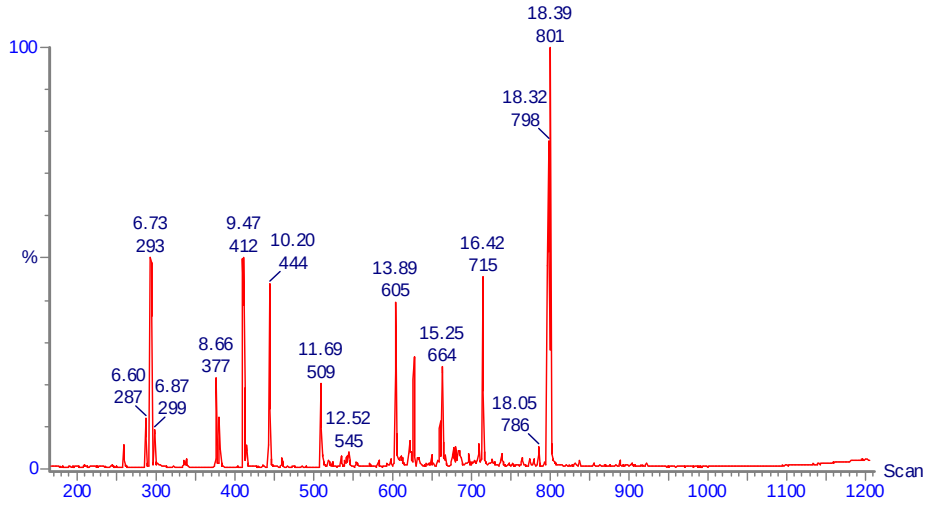
Chromatogram of sample 2 after in batch 1



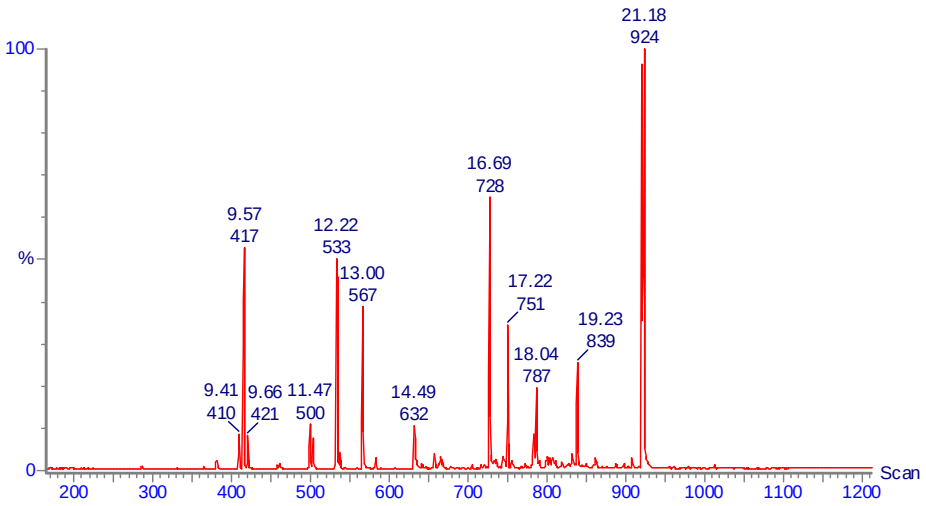
Chromatogram of sample 1 before in batch 1



Chromatogram of sample 4 after in batch 1



Chromatogram of sample 3 before in batch 1



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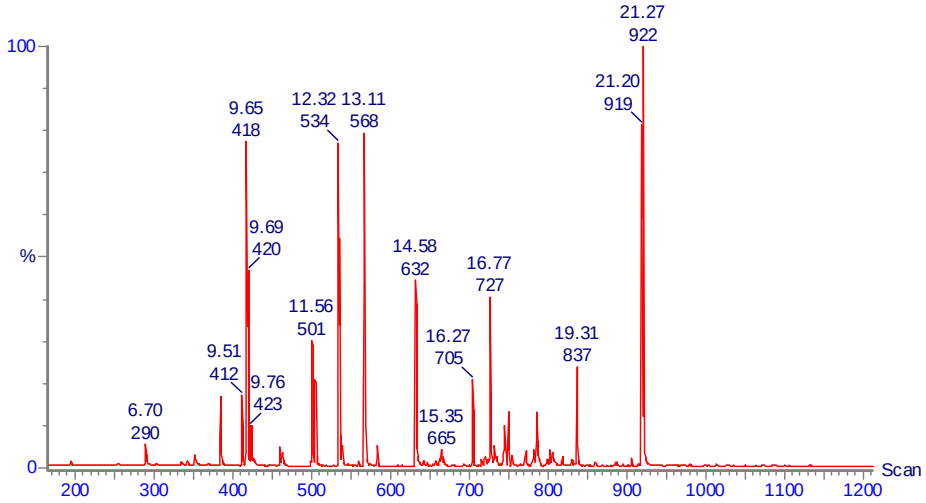
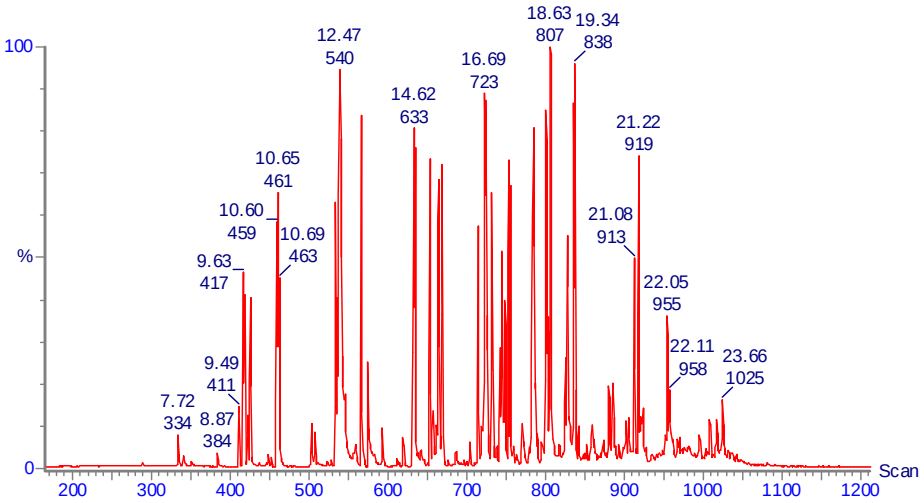
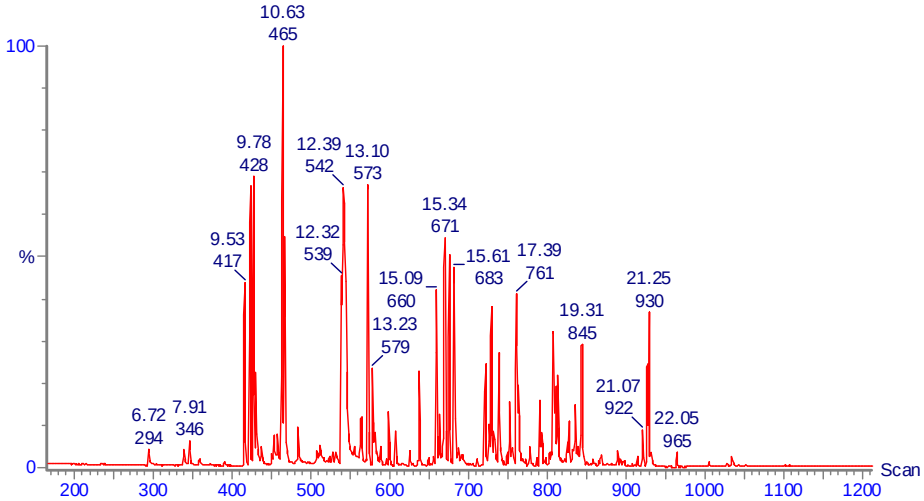


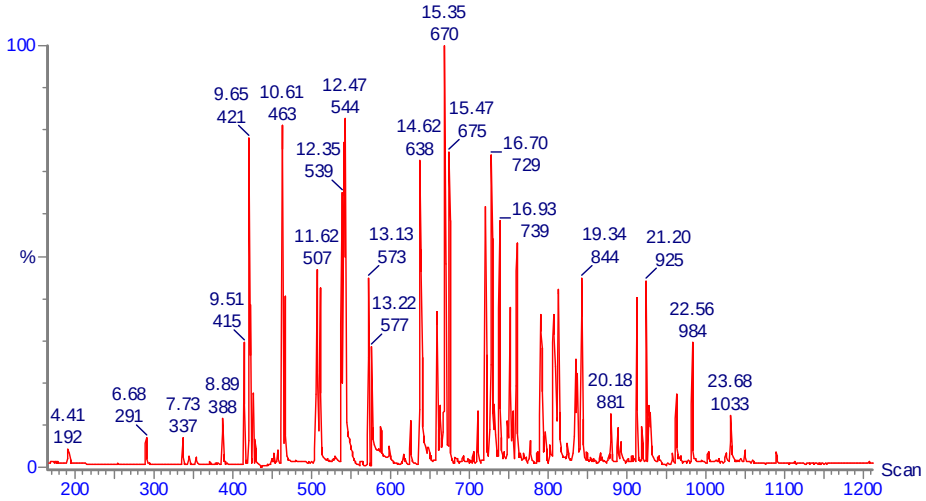
Chromatogram of sample 1 after in batch 1



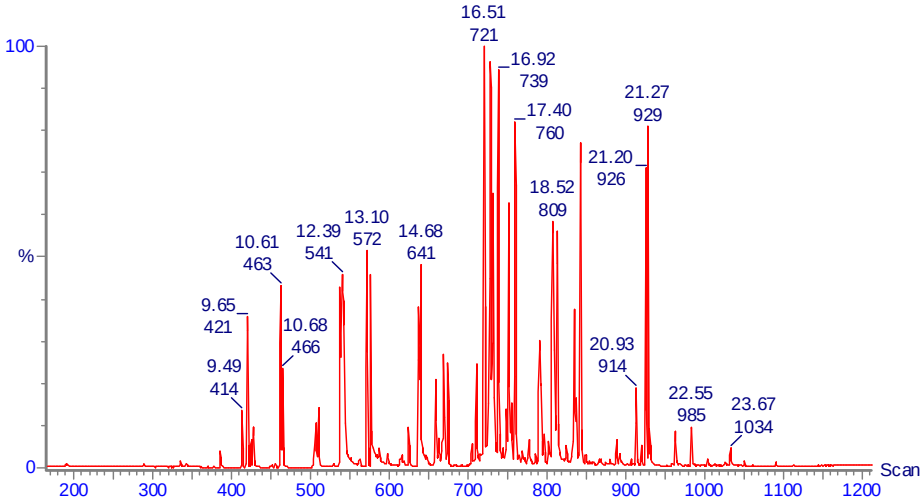
Chromatogram of Rancid (fresh almonds)



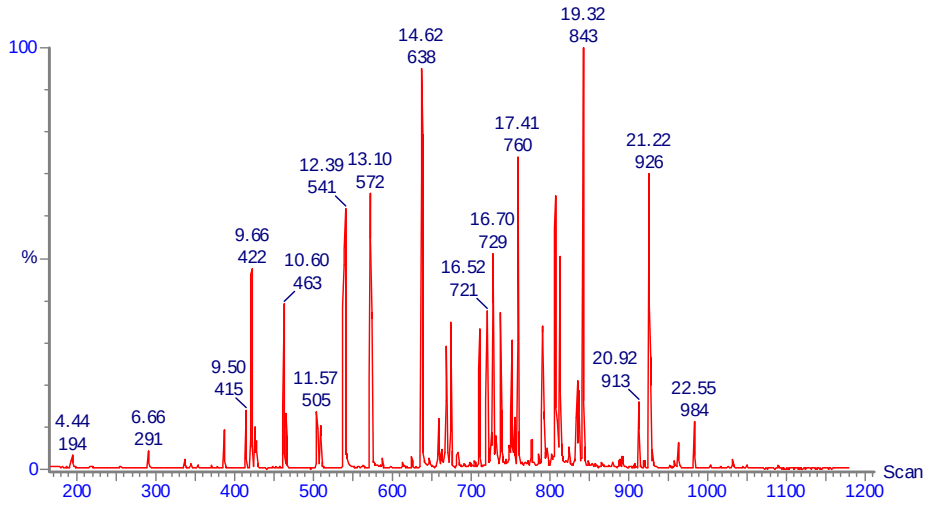
Chromatogram of Naturally oxidized sample



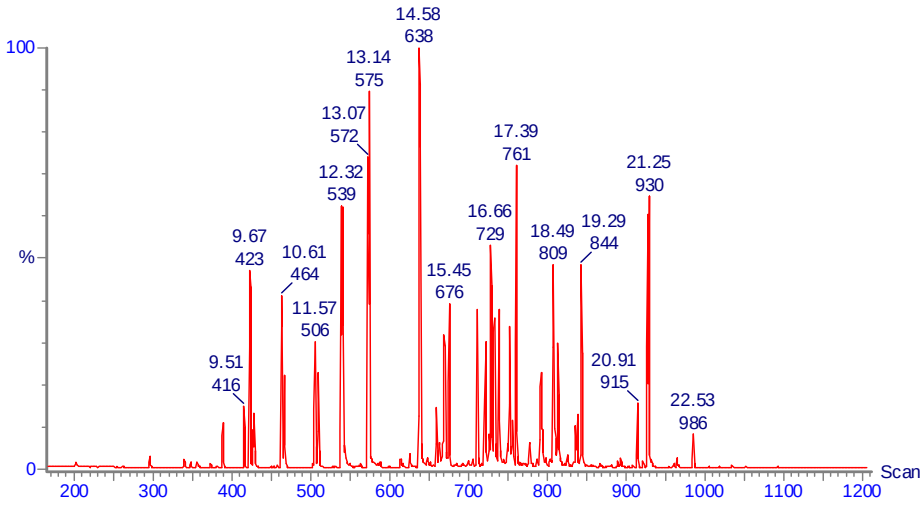
Chromatogram of sample 1 before in batch 2



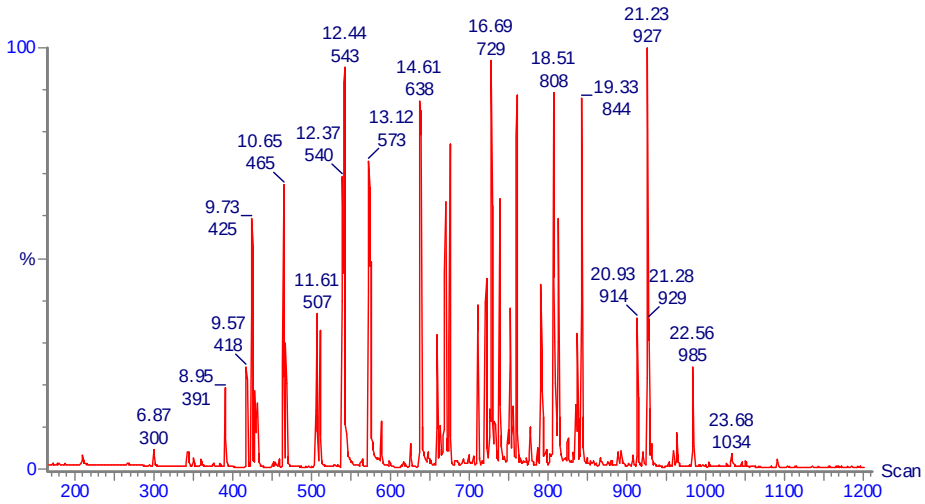
Chromatogram of sample 1 after in batch 2



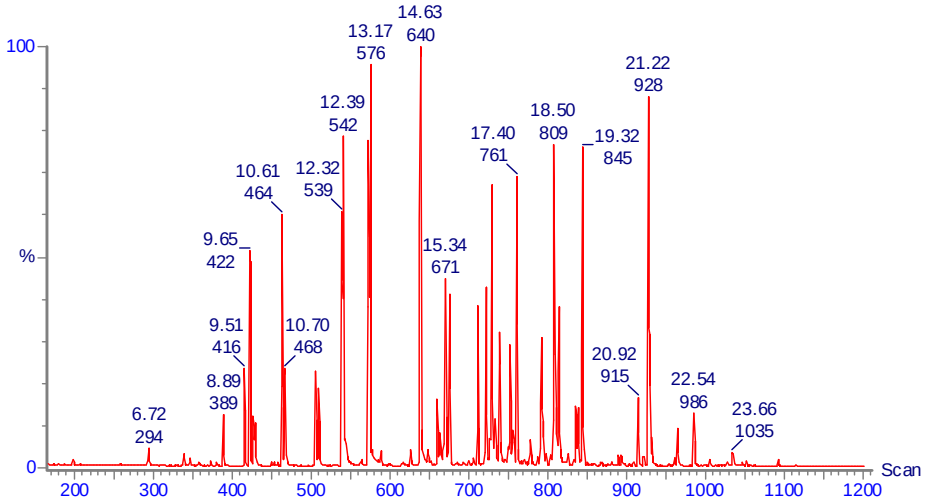
Chromatogram of sample 2 before in batch 2



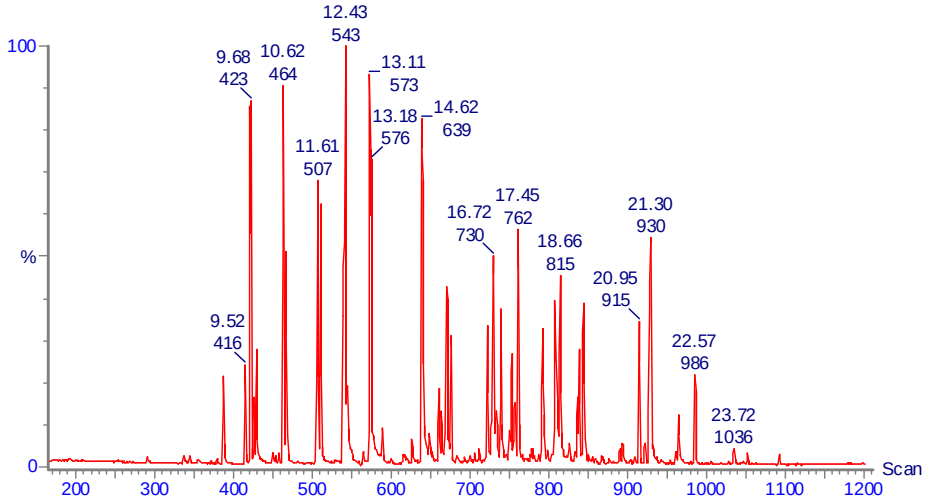
Chromatogram of sample 2 after in batch 2



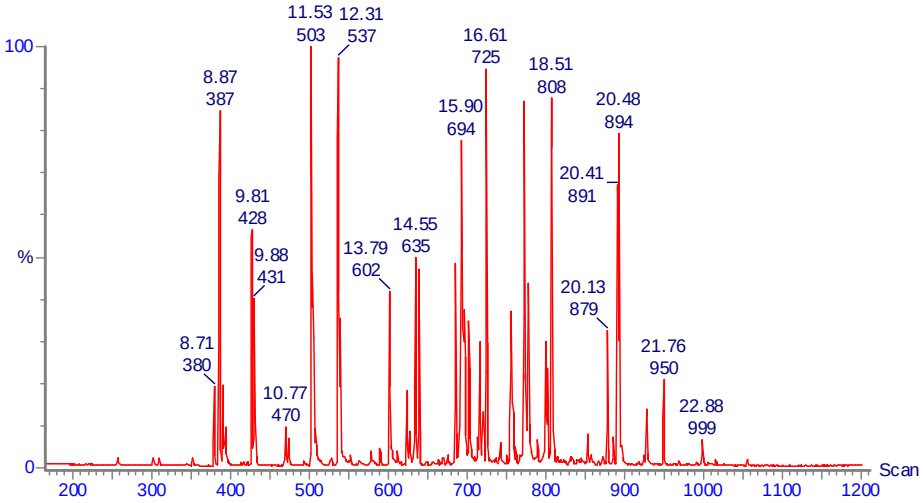
Chromatogram of sample 3 before in batch 2



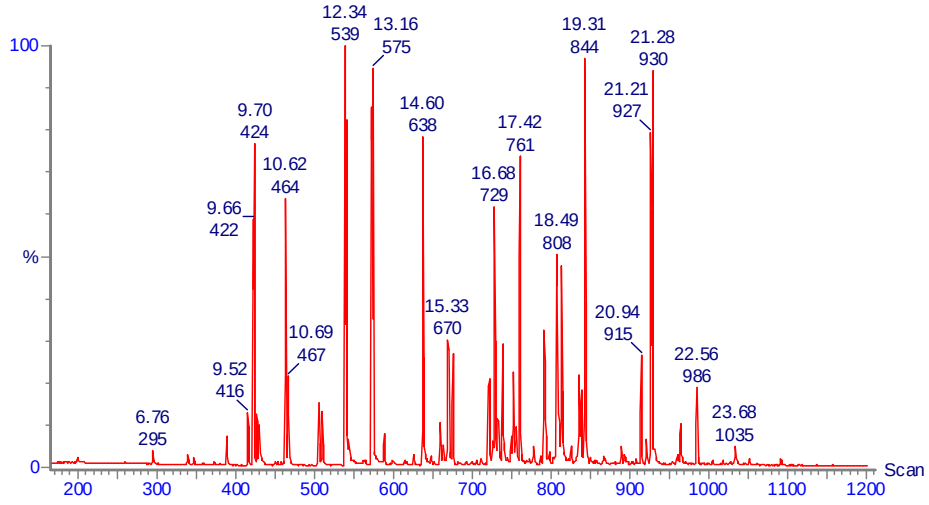
Chromatogram of sample 3 after in batch 2



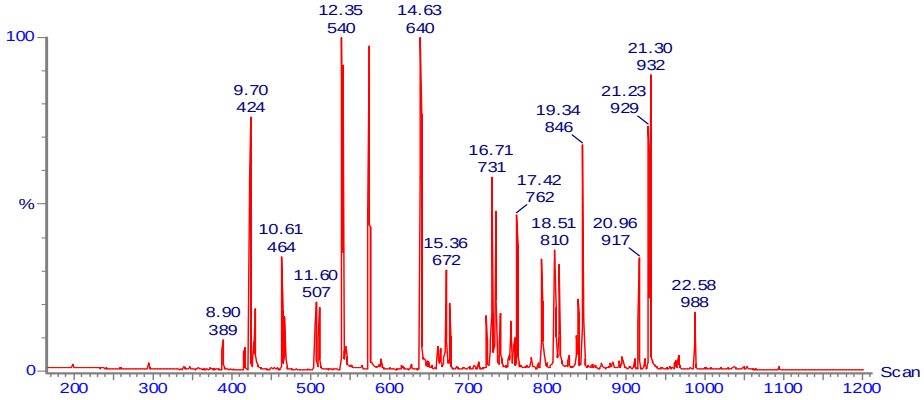
Chromatogram of sample 4 before in batch 2



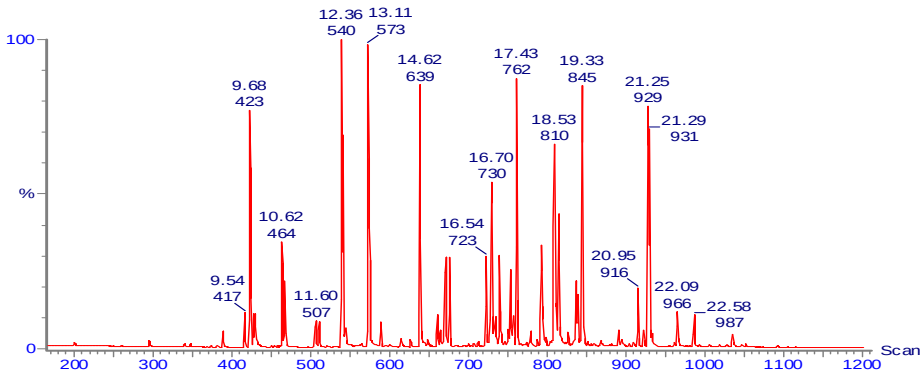
Chromatogram of sample 4 after in batch 2



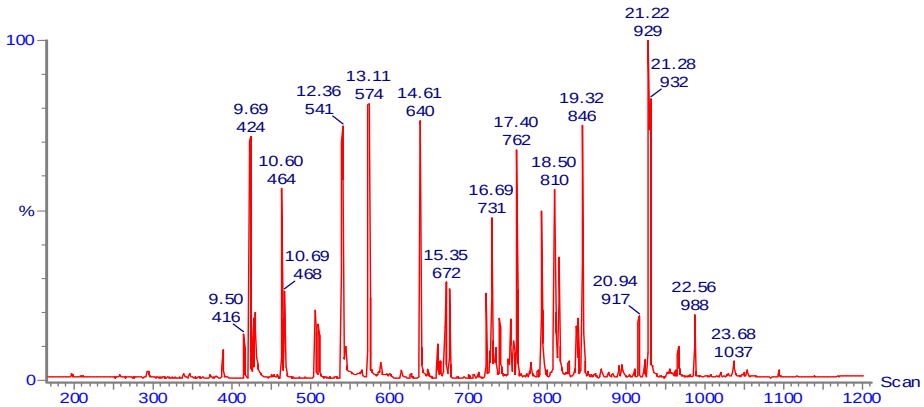
Chromatogram of sample 5 after in batch 2



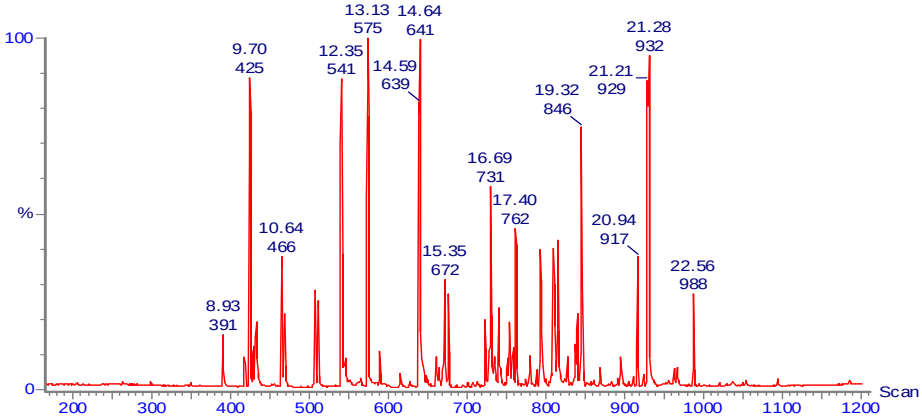
Chromatogram of sample 6 before in batch 2



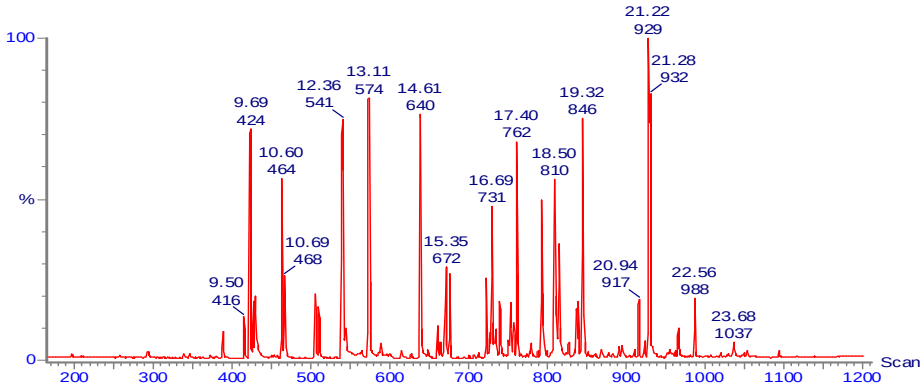
Chromatogram of sample 6 after in batch 2



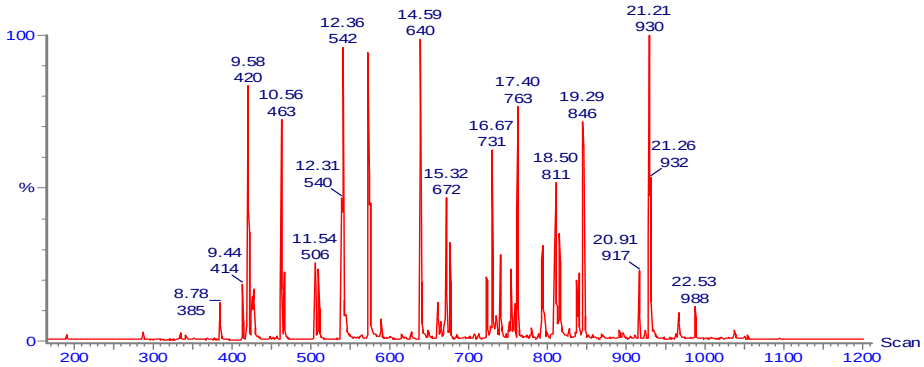
Chromatogram of sample 7 after in batch 2



Chromatogram of sample 5 before in batch 2



Chromatogram of sample 7 after in batch 2



Chromatogram of sample 5 before in batch 2

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