Date:3th May
Section:2&3

Monthly Report of Herbanol Company

Introduction

First of all, we should inform that the reports of March and April of Herbanol Company will be presented under one report. Because it was not possible that the test's results completed due to the length of one month.

This is a summary from what we have done in Feburary.According to our last report, we have used a new kind of Thyme, Zataria multiflora's (scientific name) essential oil, it is from the mint family that grows in southern Iran and has medical and edible properties.

Also, we used fresh flaxseed oil as the sample of edible oil because it is full of unsaturated fatty acids.

The process of oil's test takes time at least 25 days after we add the certain concentration of herbal essential oils to flaxseed sample .10 sample of flaxseed oil is selected and added to dark glass tubes and we put them on an oven with 40 degrees centigrade for 20 days. Two concentration 50ppm and 500 ppm of essential oil is chosen and one sample as control and another sample with synthetic antioxidant is selected.

MAIN TESTS

After 20 days the samples were refrigerated. Two main test, Peroxide Value and Thiobarbituric test have been done on samples.

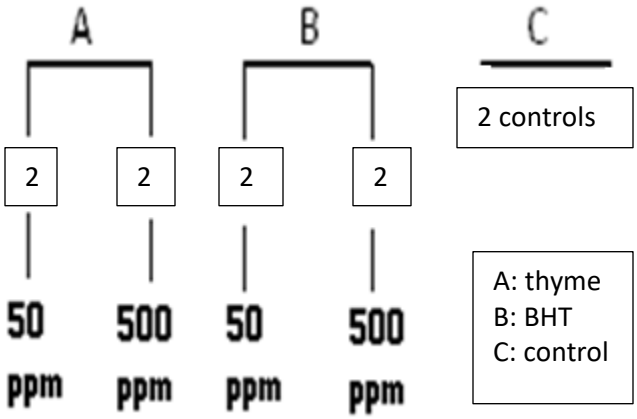
Peroxide Value

This test is done according ***the LUBRIZOL STANDARD TEST and NATIONAL IRANINAN*** test. The peroxide index is the milli equivalence of grams of active peroxide or oxygen per kilogram of oil or fat sample. The peroxide index is an indicator to show the degree of oxidative damage in oils and fats.in oxidation, peroxide or active oxygen is produced in oils. For healthy oils, this index should be below6.

This test is based on the oxidation of saturated Potassium Iodide added to the oil by oxidizing compounds in the oil such as peroxides and the formation of I2 and then the titration of Iodine released by sodium thiosulfate 0.1 N in the presence of a starch adhesive reagent.

$ROO + KI \rightarrow ROOK + \frac{1}{2}I_2$

$I_2 + Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$

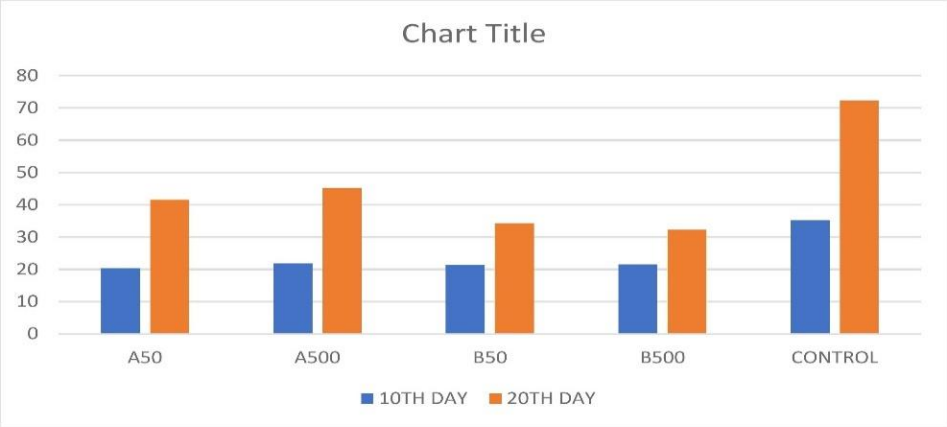


The peroxide value test has been done on 10 samples according these results.

NO.	Day	A-Max	A-Min	B-MAX	B-MIN	E-control
1	04-10-2022	21.75	20.32	21.36	21.46	35.14
2	04-20-2022	30.90	45.19	34.17	32.78	72.25

The results showed that the herbal essential oils have a significant effect on the peroxide value index compared to the control sample.

To compare the effect of adding essential oils against synthetic antioxidant BHT, a chart bar was plotted over time.



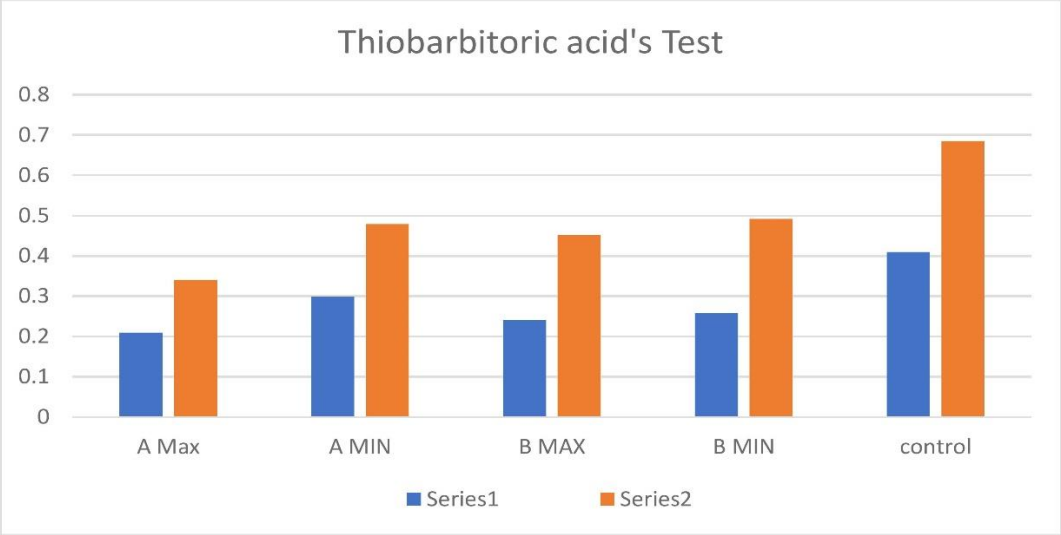
Thiobarbituric test

Thiobarbituric acid reactive substances (TBARS) are formed as a by-product of lipid oxidative damage (degradation products of fats) and can be detected by the TBARS assay using Thiobarbituric acid (TBA) as a reagent. The TBA reacts with malondialdehyde (MDA), which is one of the several low-molecular weight end products formed from the decomposition of some primary and secondary lipid peroxidation products.

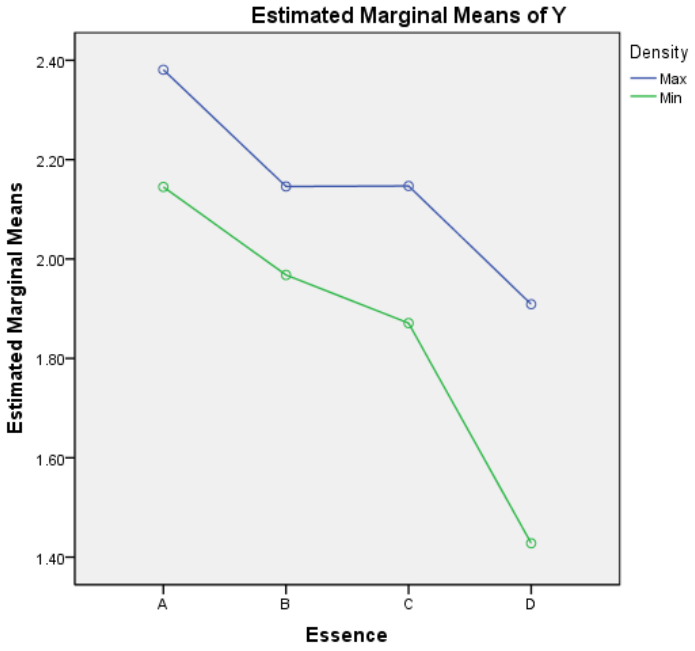
The TBA test have be done on 10 samples. And the results have been determined by UV-Vissible Bio-Tek Kontron N.

No	Days	A Max	A MIN	B MAX	B MIN	control
Series 1	**04-15-2022**	**0.209**	**0.298**	**0.240**	**0.258**	**0.410**
Series 2	**04-25-2022**	**0.340**	**0.478**	**0.452**	**0.491**	**0.685**

As the results show, the decomposition process according to Thiobarbituric acid is increasing in each period. To compare the effect of adding essential oils against synthetic antioxidant BHT, a chart bar was plotted over time.



The highest absorption is related to control sample without the presence of antioxidants. Other samples have slowed down the oxidation process to some extend due to the amount of active plant substances in them.

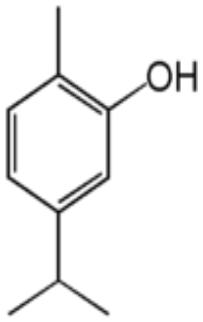
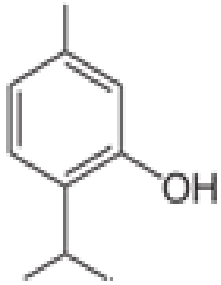
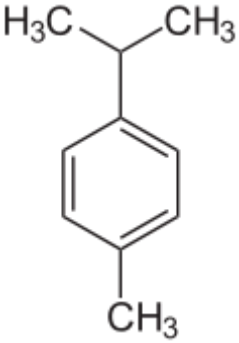


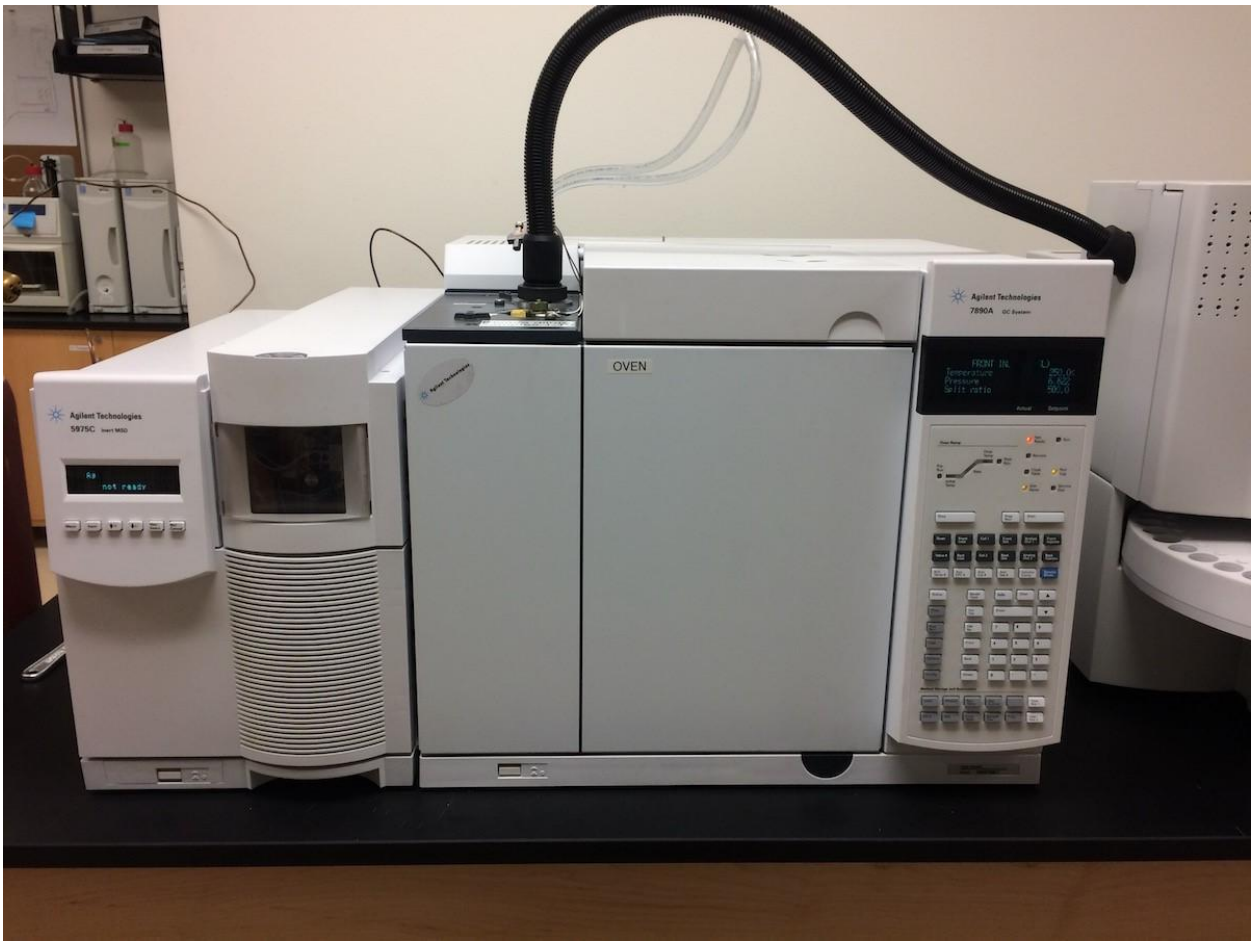
This diagram shows the simultaneous effect of concentration and type of antioxidant on oil stability. Since these two graphs do not intersect, there is no reason why there should be no interaction between type of antioxidant and the concentration.

The result of a new kind of Thyme, Zataria multiflora's (scientific name) essential oil

In addition to oral consumption as a flavoring, this plant is used in treatment of gastrointestinal disorders, local wounds and respiratory disorders and colds. As a result of analysis of thyme essential oil with GS/MS show,26 major compounds were identified.

The most important of these compounds were CARVACROL (50.22), THYMOL (21.85), PARACYMAN (6.34), BETA CARIOPHENYLENE (1.85) and LINALOOL (1.08).

		
Carvacrol(50.22%)	Thymol(21.85%)	P-cymen(6.34%)



Column type DB-1, column length 30 m, inner diameter 0.25 mm, static phase layer thickness 0.25 µm oven 40 ° C, stop at this temperature for 15 minutes, increase the temperature to 230 ° C with temperature gradient (℃) ⁄min 4, helium gas velocity 0.5 ml / min, carrier gas pressure / 69481 psi, analysis time 62.5 min, split method with a ratio of 1 to 500

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Row	Composition	Retention Index	Percentage
1	α-Tujene	16.85	0.2
2	α- pinene	17.30	0.3
3	camphene	18.11	0.2
4	β −pinene	19.67	0.48
5	3-octanone	20.06	0.71
6	β-myrcene	20.13	1.00
7	α- Terpinen	21.73	0.44
8	p-cymene	22.48	6.34
9	limonene	22.65	0.99
10	1,8-cineol	22.75	1.03
11	γ-terpinene	24.11	0.33
12	Sabinene	25.82	0.27
13	linalool	26.39	1.08
14	Borneol	29.90	0.29
15	Terpinen-4-ol	30.42	0.80
16	α- Terpineol	31.35	1.65
17	Thymol methyl ether	33.02	0.41
18	Carvacrol methyl ether	33.36	2.85
19	Thymol	35.96	21.85
20	Carvacrol	36.25	50.22
21	β-caryophyllene	42.01	1.85
22	Aromadendrene	42.30	1.40
23	β-Humulene	45.05	1.00
24	Spathulenole	48.56	0.45
25	Caryophyllene oxide	48.81	1.20
26	Aromadendrene	42.38	1.40
27	Total		98.74

Results of flaxseed oil analysis with GC / MS device

Column model: DB-WAX with a column length of 30 m, internal diameter of 0.25 mm, static phase layer thickness of 0.25 µm, oven temperature of 65 ° C and stop at this temperature for 12 minutes, temperature increase to 235 ° C, gas velocity Helium 0.5 ml per minute, carrier gas pressure 0.69481 psi, analysis time 40 minutes, split method with a ratio of 1 to 500.

This experiment was performed in two stages. In the first step, a sample of pure flaxseed oil and an oil sample that was alternately placed in the oven for 12 days were selected. The purpose of the first stage of this experiment was to identify the major constituents of the oil and also to study the trend of changes in the saturated fatty acids of the oil under the conditions of intensification of oxidation.
During the experiment by gas chromatography (GC / MS) six important and major types of fatty acids were identified and the percentage of each of them was determined. The following chromatograms are related to the analysis of these two samples. It should be noted that each of the measurements was performed with three repetitions.

The results of the analysis of two samples of flaxseed oil are as follows:

Samples	Percentage of saturated fatty acids			Percentage of monounsaturated fatty acids	Percentage of polyunsaturated fatty acids	
	C16:0	C18:0	C20:0	C18:1	C18:2	C18:3
Pure Flaxseed oil	3.86	2.48	0.13	16.03	15.94	59.05
Flaxseed oil after 20 days in the oven	3.58	2.32	0.13	14.00	13.99	56.50

The most important fatty acids in flaxseed oil are:

Saturated fatty acids:

1- Palmitic acid (C16:0)
2- Stearic acid (C18:0)
3- Arachidic acid (C20:0)

Unsaturated fatty acids:

4- Oleic acid (C18:1)
5- Linoleic acid (C18:2)
6- Linoleic acid (C18:3)

As can be seen from the above table, the percentage of saturated fatty acids during the oxidation of the oil under the condition of intensification of oxidation (high temperature) has not changed significantly because in their structures there are not double or triple bounds

However, a significant difference in the percentage of unsaturated fatty acids during the stability of the oil indicates severe oxidation of the oil and a change in the structure of the double bonds in the unsaturated fatty acids. Which is ultimately the main cause of oxidation of unsaturated oils.

This report was prepared on May 3, 2022 by Maryam Sanjari (Head of group) in collaboration with other Team Members.

Regards

Maryam Sanjari