Journal of the Advances in Agricultural Researches (JAAR) Volume: 27 (2)

Improving the Thermoxidative Stability of Soybean Oil Using Some Herbal Extracts

Mohamed Ahmed Hassan[1], Omayma EL-Sayed Shaltout[2], Ayman Abo Elyazid[2]

[1]OILEX, International Multi Oil Extraction Company, [2]Food Science Department, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria, Egypt. P.O.Box.21531

DOI: 10.21608/JALEXU.2022.134994.1061

Article Information

Received:April 20th 2022

Revised: June 9th 2022

Accepted: June 19th 2022

Published: June 31st 2022

ABSTRACT: Soybean oil is considered one of the world's major oil seed crop. Refined and deodorized soybean oil is used extensively in frying oils, salad oils and many other foods. In spite of the technological treatments of the oil still it contains 84.53% of unsaturated fatty acids which make it prone to autoxidation and the development of rancidity. Thus, the present study aims at reducing oxidation by using natural antioxidants extracted from herbs rather than using the synthetic antioxidant TBHQ which may cause health hazards and toxicological concerns. General quality tests and fatty acid composition of the refined soybean oil used in the present study was assessed. Rosemary, thyme and sage were extracted using Clevenger apparatus. Yield, total phenolic and flavonoids were measured in the herbal extracts. The antioxidant activity of the extracts was measured by two methods (DPPH and FRAP). The herbal extracts were introduced at 400 ppm to soy oil in the presence of a control and a synthetic antioxidant TBHQ at 150 ppm. Oven test was used for accelerating oxidation (180 ºC ±5 C for 18hrs at 3hrs interval) and results were tested by measuring acid value, peroxide value, iodine value, CD and CT, para Ansidine value and total oxidation (TOTOX value). Shelf life estimation was also measured using Anton's bar rancidity device. Results of the oven test showed promising results for essential oils added to soybean oil as compared to control and TBHQ where rosemary showed a clear inhibition of oxidation in secondary oxidation tests (acid value and para Ansidine) whereas, thyme and sage showed more oxidation inhibition in the primary oxidation tests (peroxide value, CD, CT and iodine value). Calculation of shelf life of samples showed best results at 20 ºC up to 30 ºC but all decreased at 40 ºC under the conditions used in the apparatus. Sensory evaluation tests after18hrs of heating were acceptable especially for rosemary and thyme. As for the fresh state all samples were acceptable with no significant differences between them.

Keywords: Aromatic plants, lipid oxidation, thermo-oxidative stability, rosemary, thyme, sage, soybean oil

INTRODUCTION

Soybean oil is derived from soybean seeds (*Glycine max*), which are farmed in a variety of countries across the world. Soybeans are one of the most valuable crops on the planet, and they are known for their versatility **(Masuda and Goldsmith., 2009).** It's one of the most widely used and widely edible oils for cooking, baking, and salad preparation around the world. This vegetable oil is high in polyunsaturated and monounsaturated fatty acids (PUFA, MUFA), low in saturated fatty acids, and free of trans fatty acids **(Anwar *et al.*, 2007).** It also includes a substantial amount of unsaturated acids such as -linolenic acid (omega 3 acid), lenolic and arachidonic acid (omega6), and oleic acid (omega 9 acid), all of which are vital in human nutrition **(Nikolić *et al.*, 2009).**

Despite the fact that soybean oil retains its natural antioxidant compounds and has a good nutritional profile due to its high level of unsaturated fatty acids, it is highly susceptible to lipid oxidation, particularly during high-temperature processing, which is unique to some food thermal applications **(Steenson and Min., 2000).** Lipid oxidation is the most common cause of oil quality degradation due to rancid and unpleasant tastes, as well as the reactive oxygen species produced, which play a key role in carcinogenesis, cardiovascular disease, and ageing processes **(Poiana *et al.*, 2016).** The chemical, nutritional, and sensory qualities of edible oils are influenced by lipid oxidation, which has a crucial role in defining the shelf-life of edible oils and ultimately their uses **(Dobarganes and Márquez-Ruiz, 2003).** Sensory and molecular changes as well as, a decline in nutritional content may occur as a result of oxidation. Furthermore, it causes the formation of potentially hazardous dimers, polymers and cyclic monomers **(Juárez *et al.*, 2005).**

Antioxidants are added to food to delay, minimize or avoid oxidative degradation. Some synthetic antioxidants used in food processing, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene, propyl gallate and tertiary butyl hydroquinone (TBHQ), have had their side effects investigated and their use has been questioned due to toxicological concerns **(Wang *et al.*, 2008).** Several investigations have been conducted to discover natural compounds with antioxidant activity **(Proestos *et al.*, 2010).** Many compounds found in plants, including herbs and spices, have been identified as possible sources of natural antioxidants **(Peng *et al*. 2005; Zhang *et al.*, 2010).** Spices and aromatic herbs are gaining popularity in the industry and in scientific study due to their powerful antioxidant and antibacterial characteristics, which outperform many commonly employed natural and synthetic antioxidants **(Al-Dalain *et al*., 2011).**

Many foods contain synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary butyl hydroquinone (TBHQ). However, their use has been questioned because to concerns about toxicity and carcinogenicity. As a result, natural antioxidants in foods have received a lot of attention because of their potential nutritional and therapeutic advantages **(Gamez-Meza, *et al.*, 1999).** The food industry is becoming increasingly interested in crude extracts of herbs and spices, as well as other plant materials high in phenolics, because they delay oxidative lipid destruction and hence increase the quality and nutritional value of food **(Al-Dalain *et al*., 2011).**

The aim of this study was to evaluate the effect of essential oils extracted from rosemary, thyme and sage on the thermoxidative stability of soybean oil using the heat treatment (at 180 ºC ±5 C for 18 h at intervals of 3 h heating for 6 consecutive days) in closed oven. Then subjected to analytical tests. Samples are also subjected to Anton bar's rapidoxy device for calculation the shelf life of different samples.

MATERIALS & METHODS

Refined Soybean Oil was purchased from Oilex Company in El Sadat City in El Menoufia Governorate, Egypt.

Rosemary Leaves, Sage and Thyme Leaves were purchased from a specialized market for aromatic & medicinal plants (Awlad Ragab) in Cairo city.

Synthetic antioxidant Tertiary butylhydroquinone (TBHQ) from IUNA company for fragrances and flavour (Egyptian company)

Chemicals: All Chemicals were purchased from Scharlau company for laboratory chemicals and microbiological cultures, Barcelona, Spain.

Extraction of essential oils (natural antioxidants): Essential oils of the three herbal plants under study were extracted by water distillation (hydro-distillation) using Clevenger type apparatus according to the method of **Guenther (1952).**

Analytical tests of essential oils:

Determination of total phenolic compounds (TP): Folin-Ciocalteu spectrophotometric method as described by **Al-Owaisi *et al.*, (2014)** was used to determine the total phenolic content in the essential oils. Total phenolic content was determined using a linear regression equation obtained from the standard plot of gallic acid and expressed as mg gallic acid equivalent (GAE)/g of extract.

Determination of total flavonoids compounds: Total flavonoids content in the aqueous and ethanolic extracts were determined spectrophotometrically by the method of **Zarina and Tan(2013**). Rutin (20- 100 µg /ml) was used as standard by which a calibration curve was obtained. Total flavonoids content was expressed as mg rutin equivalent /g extract or dry part.

Determination of antioxidant activity:

Scavenging Activity of DPPH Radical: The anti-radical activities of oil extract were determine based on a reaction with stable 2,2-diphenil-1-picrylhydrazyl (DPPH) radical dissolved in absolute methanol **(Shehata *et al*.,2017)** The reduction of DPPH with an antioxidant decreases the absorbance of the solution at 517 nm. The reaction requires preparing a mixture of 500 µl of oil at various concentration with 375 µl of methanol and 125 µl of DPPH solution (0.02% prepared in methanol). For each concentration, a blank is prepared by mixing 500 µl of the sample with 500 µl of methanol. A control containing 875 µl of methanol and 125 µl of DPPH solution was also prepared. After incubation for 60 min in the dark, the absorbance at 517 nm was measured. The anti-radical activity was determined using the following formula: The inhibition of DPPH radical % = (Abs control – Abs sample)/Abs sample X100 Notably, a lower absorbance by the reaction mixture indicated a higher DPPH scavenging activity.

Ferric reducing antioxidant power (FRAB) assay: The reducing power of ethanolic extracts was according to the method cited by **Oyaizu (1986)** which involves the presesnce of antioxidants in extract to reduce the ferricyanide complex to the ferrous form. One milliter of extract in different dilutions was added to 2.5 ml phosphate buffer (0.1 M, pH 6.6 and 2.5 ml potassium ferricyanide (1% w/v). The mixture was then incubated in a water bath at 50ºC for 20 min followed by 2.5 ml tricholoacetic acid (10% w/v) solution. The contents of the tubes were mixed well and 2.5 ml of solution was removed from each tube. To this, 2.5 ml solution, 2.5 ml water and 0.5 ml ferric chloride solution (0.1% w/v) were added. The mixtures were allowed to stand for 30 min before absorbance measurement were taken at 700 nm. Triplicate tubes were prepared for each extract. Increased absorbance of the reaction mixture indicated increased reducing power.

Sample Preparation: The oil was divided into 5 samples. The control sample and the other samples containing different types of essential oils and a synthetic antioxidant as shown in table 1.

Table (1) Herbal essential oils and synthetic antioxidants added to soybean oil

Soybean oil (control)	-	SBOC
Soybean oil + Rosemary essential oil	400 ppm	SBOR
Soybean oil + Thyme essential oil	400 ppm	SBOTH
Soybean oil + Sage essential oil	400 ppm	SBOS
Soybean oil + TBHQ	150 ppm	SBOTB

Mixing soybean oil with natural antioxidants is according to the recommended level described in **(Al-Dalain *et al.,* 2011).**

Oven test: All soybean oil samples were heated at 180 ºC ±5 C for 18 h at intervals of 3h heating for 6 consecutive days in closed oven. The heated oils were analyzed every day after heating then put in brown bottles and kept at 5 ºC for 24h for further analytical tests.

Analytical tests of mixing soybean oil

Fatty acids composition: The fatty acid methyl esters of refined soybean oil was prepared using trans-esterification with cold methanolic solution of sodium hydroxide. The fatty acid methyl esters were identified by Gas-Chromatographic analysis of lipids in biological materials according to **(Petrović *et al*., 2010).**

Determination of Free Fatty Acids (FFA): according to AOCS AC 5-41 (2009).

Determination of Peroxide Value (P.V): according to AOCS Cd 8b-90 (2011).

Determination of Iodine Value (I.V): according to AOCS Cd 1-25 (1998).

Determination of P.Ansidine: according to AOCS Cd 18-90 (2011).

Determination of Conjugated Diene & Conjugated Triene: according to IUPAC (1987).

Determination of Total Oxidation (TOTOX): TOTOX value was calculated according to formula:

TOTOX value = 2 * PV + pAV

Determination of the shelf life of soybean oil mixed with different antioxidant by rapidoxy device:

The Rapidoxy 100 artificially accelerates the oxidation process by using increased temperature and oxygen pressure. As a general rule, samples with a shorter induction period are less stable than samples with a longer induction period. Results are measured by establishing a regression equation between pressure, temperature and time. At the test, we use two programs. First one at 100°c at pressure 7 bar and the device record its time. Second one at 120°c at pressure 7 bar. During each test, the device takes records for temperature, pressure and time to use it to make the regression equation which will be more easier to us to change variables as we want like we want the result be estimated at 1 pressure and the temperature be 25°c. The method gives you a multiple choices of temperature to change with high prescion and accuracy because of the many points recorded during test which will give a powerful high R-square value.

Statistical analysis: Data were analyzed by using analysis of variance (ANOVA) according to the General Linear Models Procedure (GLM) of the SAS package (SAS Institute, Inc.,1994). Comparison of treatment means and time were further differentiated using Walter-Duncan's multiple range test at P (<0.01)

RESULTS & DISCUSSION:

The initial characteristics of soybean oil: The initial characteristics of refined soybean oil used in the present study are given in Table 2. Tabulated data revealed that the soybean oil is of very good quality, as indicated by its initial low characteristics. The chemical characteristics of soybean oil established its capability of application in either nutrition or industry.

Fatty acid composition of soybean oil: Table (3) represent the fatty acid composition of soybean oil used in the present study.

Results showed that the highest saturated fatty acid is palmitic acid C16:0 (10.69%) whereas, the highest unsaturated fatty acid is linoleic acid C18:2 (54.97%). Linolenic acid was 7.9% which was less than that reported by **Almoselhy (2021)**, whereas, linoleic acid and oleic acid were within the range reported by **Park& Hyunwoo, (2012)** who reported that they were 55% and 18% of total fatty acids, respectively. It's worth mentioning that the major unsaturated fatty acid in soybean oil is the polyunsaturated alpha-linolenic acid (C18:3n) which usually range between 7-10%. It was mentioned by **(Clemente and Cahoon, 2009)** that at this level oxidative stability of the oil could be reduced which can lead to rancidity and decreased shelf life. The ratio between saturated and unsaturated fatty acid in many fats and oils is used as a quality indicator where the ratio increase with the increase of number of frying. As a comparison the SFA/USFA for fresh flaxseed oil is 0.099 compared to 0.183 for fresh soybean oil in the present study. As a matter of fact, it's worth mentioning that the fatty acid profile depends on the genotype.

Table (2) Chemical analysis of soybean oil

CHARACTERISTICS OF SOYBEAN OIL	
FFA % (AS OLEIC ACID)	0.019
IODINE NUMBER	131.1
PEROXIDE VALUE (MEQ/KG)	0.075
P-ANSIDINE	0.705
CONJUGATED DIENE	0.18
CONJUGATED TRIENE	0.075

Table (3) Fatty Acids Composition of soybean oil

Fatty acid composition %	
Myristic acid C14:0	**0.2**
Palmitic acid C16:0	**10.69**
Palmitolic acid C16:1n9C	**0.08**
Margaric acid C17:0	**0.09**
Stearic acid C18:0	**4.49**
Oleic acid C18:1n9C	**21.29**
Linoleic acid C18:2n6C	**54.97**
Linolenic acid C18:3n3	**7.9**
Gendoic acid C20:1	**0.29**
SFA	**15.47**
USFA	**84.53**
SFA\USFA	**0.183**

Yield and percentage of essential oils: Table (4) shows the of essential oils obtained after distillation using Clevenger apparatus. Results revealed that with the same quantity (500 g) of herbs, thyme exhibited the highest yield of extract whereas, rosemary and sage exhibited the same yield (7ml). It was reported by **(Sayyad *et al.*, 2017).** That there is no relation between yield and antioxidant activity of the essential oils.

Table (4) Yield and percentage of essential oils:

Plant name	Quantity g	Yield ml	Percentage %
Rosemary	500	7	1.4
Thyme	500	18	3.6
Sage	500	7	1.4

Total phenolic, total flavonoids and antioxidant activity of the essential oils:

Table (5) shows the total phenolics (TP) and flavonoids (TF) in essential oils extracted as well as their antioxidant activities as measured by DPPH and FRAP. Results indicated that all essential oils contain a considerable amount of TP and TF where sage showed the highest concentration in this respect. Result also indicated that sage exhibited the highest antioxidant activity followed by thyme while rosemary came last in comparison to them, these results clarify that herbs are excellent sources of antioxidants rich in phytochemicals, which contain flavonoids and phenolic compounds all exhibit high antioxidant activity **(Srinivasan *et al* ,2014 and Embuscado, 2015).** Different antioxidant activity tests clarified the antioxidant activity of such herbs **(Ivanovic *et al*.,2013).** It has also been reported that rosemary, thyme and sage (family lamiacea) are the most potent antioxidant herbs used in food systems.

It is worth mentioning that in the present study although rosemary exhibited the lowest TP, TF, DPPH and FRAB compared to sage and thyme, this does not mean that the antioxidant power of rosemary is less effective. Reports frequently showed the power of rosemary as a good antioxidant in retarding the onset of oxidation in many foods, as well as sage and oreganes **(Sayyed *et al*.,2017, Pizzal *et al*.,2001, Zheng and Wang, 2001).**

It is worth mentioning that FRAB measure reducing power but it cannot detect compounds that act by radical quenching **(Xu and Schaich,2005),** whereas DPPH is a measurement of reducing ability of antioxidant. The DPPH radical scavenging ability uses other units in addition to% inhibition. **(Prior *et al*., 2005).** It was reported that DPPH values of clove, sage and rosemary have the highest antioxidant activities. Differences between DPPH values of the same species or herbs were due to the solvents used in extraction **(Amritha., 2014)** it was also reported the differences in total phenolics from the same herbs are due to different solvents used for extraction and possibly due to their natural variation of antioxidant activities as influenced by variety, location of growth, fertilization and weather **(Carlesen *et al*.,2010).**

Table (5) Antioxidant activity, total phenolic content and total flavonoids of the essential oils

Plant name	TPC [mg GAE/g]	TF (mg/g)	DPPH %	FRAP mM/100g
Rosemary	40.57mg/g	15.26	47.73	14.50
Thyme	56.27	21.58	56.66	55.40
Sage	96.1	28.5	67.7	83.4

Oven heat treatment of soybean oil with added essential oils: Accelerated shelf life testing is an industrial procedure to find out whether a particular oil or fat is likely to be able to withstand storage over a period. The probable course of the rancidity can be predicted by methods in which the oxidation is accelerated by passing air through the sample or by rising the temperature then measuring FFA and Peroxide value and other tests **(Egan *et al*.,1981)**

Free Fatty Acids (FFA):

Fatty acids represent an inherent part of the fat molecular structure. During heating, however, they are hydrolyzed from the oil/fat to become FFA. Hydrolysis, a chemical reaction that occurs in the presence of moisture and heat decomposes triglycerides into free fatty acids.

Elevated levels of FFA cause oils to generate smoke during frying, hasten oxidative degradation, and contribute to off-flavors **(O'Brien 1993).**

As a matter of fact, fig (1) show that both rosemary, sage and thyme exhibited lower FFA values with no significant difference between them as compared to TBHQ which exhibited the lowest value. As a matter of fact the control exhibited the highest FFA in this respect. It is well known that FFA represent a tertiary stage of oxidation, meanwhile all added essential oils from rosemary, thyme and sage showed resistance in this respect by decreasing FFA as compared to control.

It was confirmed by **Buck and Edwards (1997)** that TBHQ is still the superior single antioxidant for most vegetable oils. This result also confirm what was reported by **(Ivanovic *et al*.,2013)** that rosemary, thyme and sage (family lamiacea) are the most potent antioxidant herbs used in food systems.

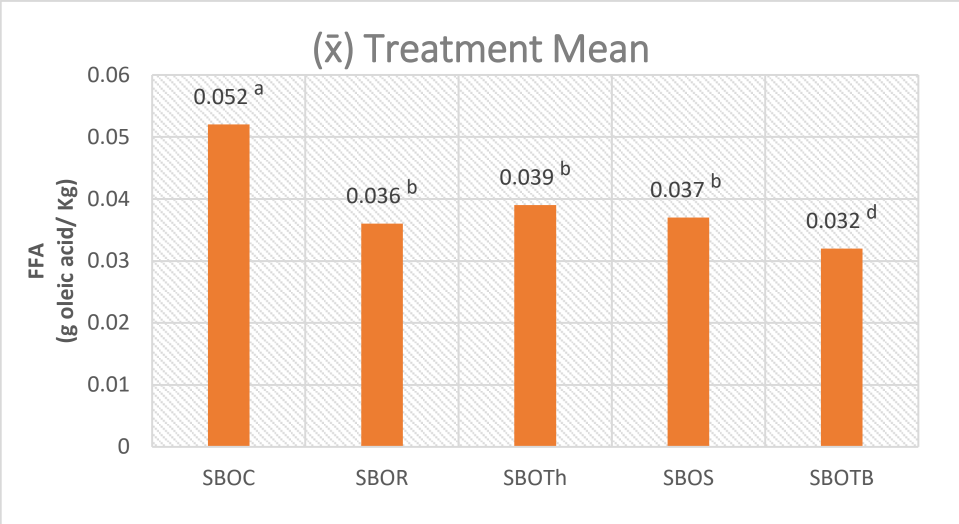

Fig (1) Effect of essential oil treatments on free fatty acid value

SBOC soybean oil sample control, **SBOR** soybean oil + rosemary essential oil, **SBOTH** soybean oil + thyme essential oil, **SBOS** soybean oil + sage essential oil, **SBOTB** Soybean oil + TBHQ.

Peroxide Value (P.V):

Lipid peroxidation is one of the main reasons for deterioration of food products during processing and storage. The peroxide value (P.V) is an indicator of the initial stages of oxidative change. **(Shahidi and Zhong., 2010).**

Results of peroxide value (PV) showed that thyme significantly reduced (P< 0.01) the rise in PV (Fig 2) under the experimental conditions followed by sage then rosemary and TBHQ as compared to control which exhibited the highest value, in this respect. Still the natural antioxidants from herbs exerts its powerful effects by lowering the onset of primary oxidation as measured by PV and compete with the synthetic antioxidant. Several studies have reported that thyme is a source of bioactive compounds. **(Nieto., 2020).** studied the essential oil content of thymus species he found that the most common essential oil was thymol and linalool. It was also reported that the antioxidant activity is related to thymol which is a better antioxidant than carvacrol.

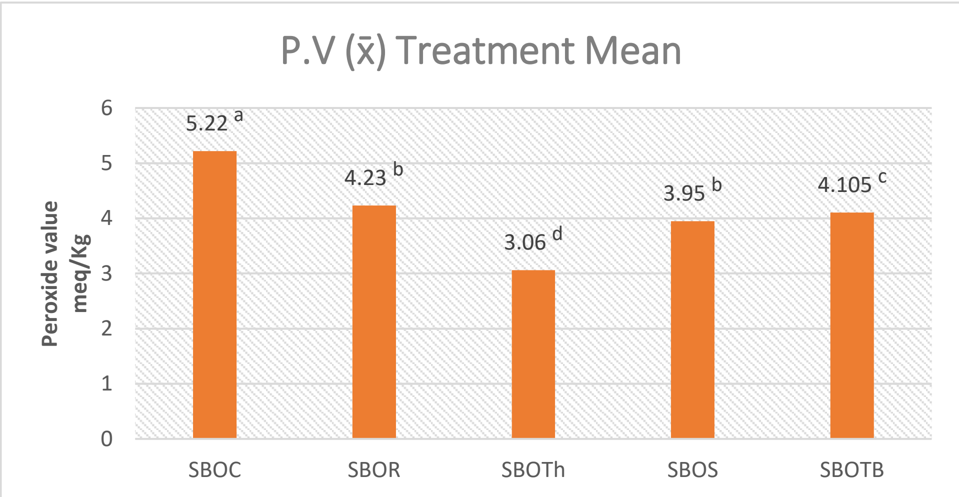

Fig (2) Effect of essential oil treatments on peroxide value

SBOC soybean oil sample control, **SBOR** soybean oil + rosemary essential oil, **SBOTH** soybean oil + thyme essential oil, **SBOS** soybean oil + sage essential oil, **SBOTB** Soybean oil + TBHQ.

Iodine Value:

The iodine value or iodine number (IV) is defined as the mass of iodine in grams that is consumed by 100 grams of a chemical substance. Iodine value is often used to determine the amount of unsaturation in fats, oils and waxes. The iodine value is also used as a measure of oxidation.

As can be seen, thyme preserved the highest IV value (figure 3) thus reducing the onset of oxidation followed by TBHQ treatment which showed no significant difference as rosemary, as a matter of fact, sage was also effective in less manner but still better than control. The superiority of thyme extract may be attributed to its components such as monoterpene phenols including carvacrol, thymol and p-cymene and other monoterpenes. It was concluded by **(Wanasundara *et al.*, 1996)** that the inhibitory capacity of oxidation of thymus species extract is fundamentally due to its richness in phenolic compounds. The antioxidant activity of these compounds are due to their ability to act as scavengers of free radicals, metal ion chelators and inhibitors of oxidative enzymes **(Nieto., 2020).** In this respect also, rosemary showed a great competition with the synthetic antioxidant in preventing the onset of oxidation as compared to control **Sayyed *et al.*, (2017)**.

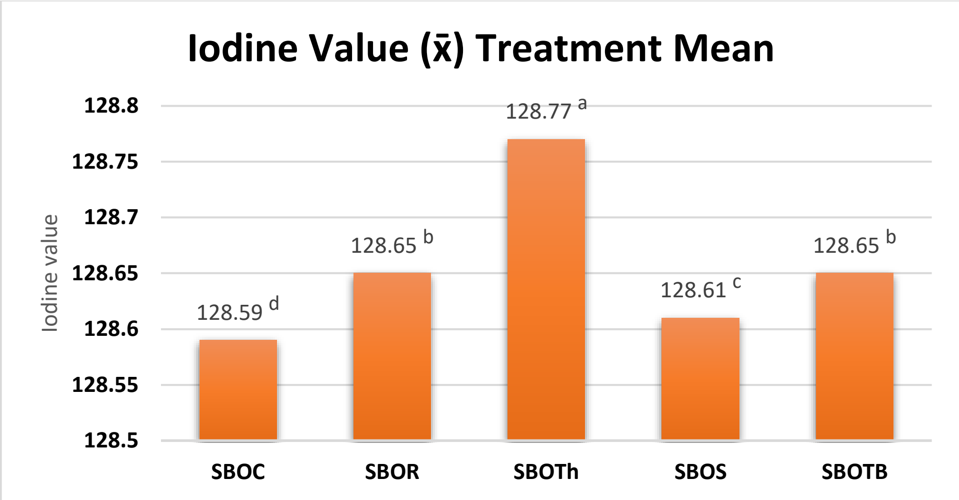

Fig (3) Effect of essential oil treatments on iodine value

SBOC soybean oil sample control, **SBOR** soybean oil + rosemary essential oil, **SBOTH** soybean oil + thyme essential oil, **SBOS** soybean oil + sage essential oil, **SBOTB** Soybean oil + TBHQ.

Para- Ansidine:

P-Ansidine value (An-V) is a method for measuring the secondary decomposition products such as aldehydes and ketones. The oxidative deterioration of lipids involves primary autoxidative reaction which is accompanied by various secondary reactions having oxidative and non-oxidative reaction. The primary products of lipid oxidation are hydroperoxides. The secondary products are aldehydes and ketones.

Results also show the effect of essential oil treatments (fig 4) on An-V. Both rosemary and TBHQ significantly exerted the least values of An-V as compared to control which showed the highest An-V in this respect. The An-V measures the compounds resulting from the breakdown of the peroxides such as alfa aldehydes. The previus results also show that all natural antioxidants rosemary, thyme and sage still can compete with the synthetic antioxidant TBHQ, where thyme and sage showed no significant differences between them.

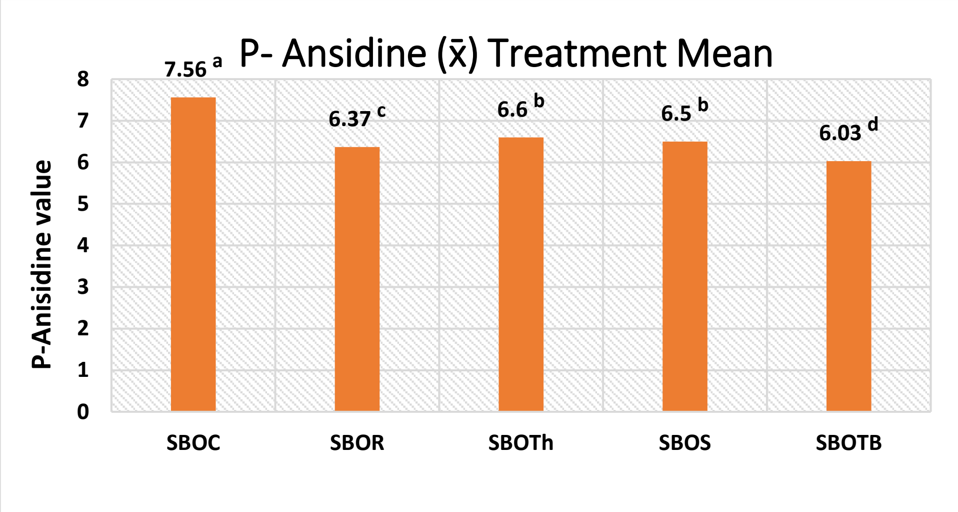

Fig (4) Effect of essential oil treatments on P.Ansidine value

SBOC soybean oil sample control, **SBOR** soybean oil + rosemary essential oil, **SBOTH** soybean oil + thyme essential oil, **SBOS** soybean oil + sage essential oil, **SBOTB** Soybean oil + TBHQ.

Conjugated dienes:

Conjugated dienes and trienes are a good measure of primary oxidation of the oil. Results also clarified the effect of different EO treatments which elucidated the superiority of thyme and sage followed by TBHQ and rosemary in decreasing the CD values which can be organized in the following descending arrangement: thyme > sage > TBHQ > rosemary > control. The formed CD exhibited intense absorption at 234nm, due to a shift in the double-bond position of lipids containing methylene interrupted dienes during oxidation. The absorption increase is proportional to the uptake of oxygen and formation of peroxide in early stages of oxidation **(Longani and Davis.,1980)**

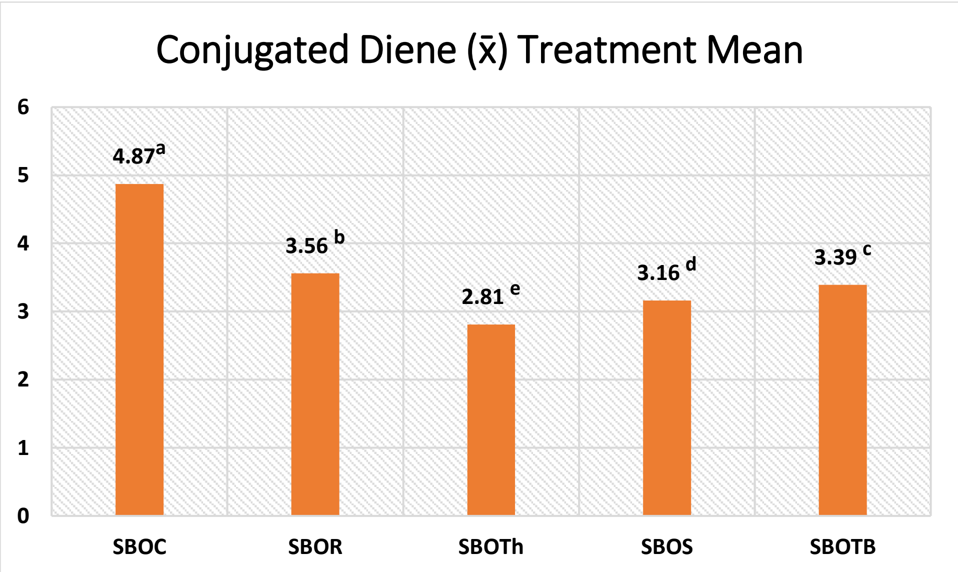

Fig (5) Effect of essential oil treatments on conjugated diene value

SBOC soybean oil sample control, **SBOR** soybean oil + rosemary essential oil, **SBOTH** soybean oil + thyme essential oil, **SBOS** soybean oil + sage essential oil, **SBOTB** Soybean oil + TBHQ.

Conjugated triene:

Both conjugated dienes and trienes measure primary lipid oxidation. Figure (6) also shows the effect of the different natural herbal extracts in lowering the onset of oxidation. Both thyme and sage exhibited significant lower values with no significant differences between them and were less than the synthetic TBHQ and rosemary whereas the control recorded the highest CT value.

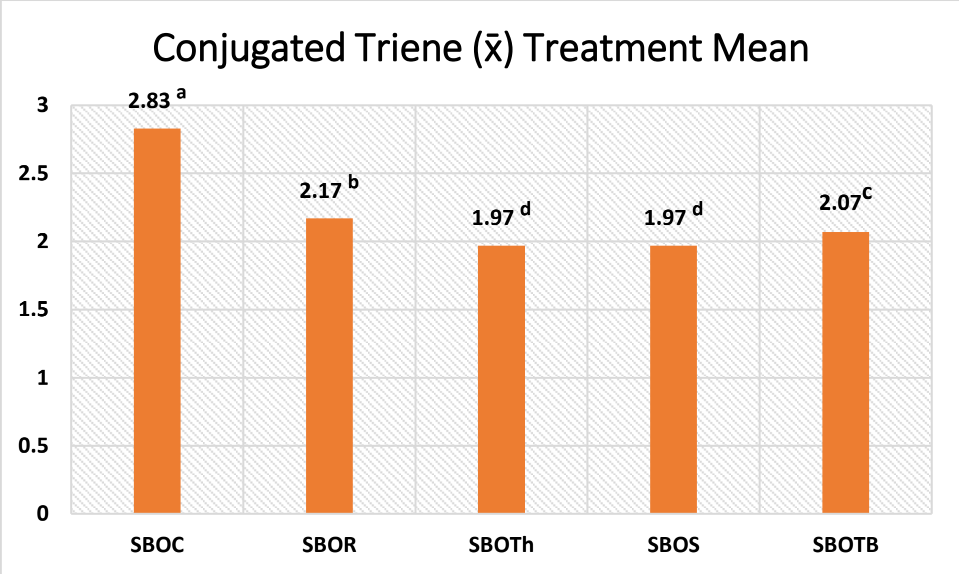

Fig (6) Effect of essential oil treatments on conjugated triene value

SBOC soybean oil sample control, **SBOR** soybean oil + rosemary essential oil, **SBOTH** soybean oil + thyme essential oil, **SBOS** soybean oil + sage essential oil, **SBOTB** Soybean oil + TBHQ.

TOTOX value (Total Oxidation):

TOTOX is abbreviation for total oxidation value which is a number to describe the total oxidation load to which a fat has been exposed, or it gives a picture of total oxidation deterioration of an oil or fat.

As a matter of fact, thyme exhibited the least TOTOX value in this respect followed by TBHQ. Control recorded the highest value in this respect. In a comparison of the antioxidant activity between thyme and other antioxidant compounds, thyme was reported to be one of the best antioxidants. This may be due to the discovery and identification of a new component p-cimen-2,3-diol, isolated for the first time from thyme leaves, which showed higher antioxidant activity than that of α-tocopherol and BHA **(Nieto., 2020).** Sage likes thyme, contains a number of powerful essential oils that have strong antioxidant properties. Such as Thujone (19.89%), camphor (15.12%), borneol (12.89%), and 1.8-cineole (12.06 percent) are some of the primary essential oils found in sage **(Moura-Alves and colleagues, 2020)** However, **(Youdim *et al.*, 2002)** carried out a study on the antioxidant properties of the essential oils of thymus zygis and its major components, confirming that the effectiveness of these substances increased in the following order:

Essential oil > thymol > carvacrol > β-terpinene >mircene > linalool > p-cirmene > limonene > 1,8-cineool > α-pinene. Accordingly, all the tested compounds have antioxidant activity .

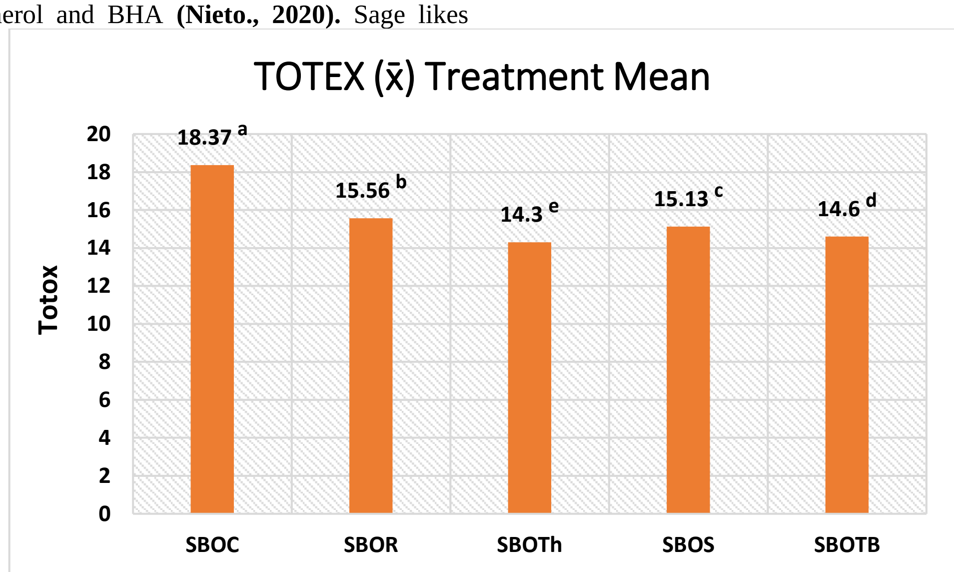

Fig (7) Effect of essential oil treatments on totox value

SBOC soybean oil sample control, **SBOR** soybean oil + rosemary essential oil, **SBOTH** soybean oil + thyme essential oil, **SBOS** soybean oil + sage essential oil, **SBOTB** Soybean oil + TBHQ.

Shelf life expectancy using Rapidoxy device:

Results show (fig 8) that at 20°C, the shelf life of the studied oils can be sorted as follows: SBOTB > SBOS > SBOTH > SBOR > SBOC, with 540 days, 464 days, 447 days, 412 days, and finally 405 days for control, respectively. At 25°C, however, the shelf life expectancy decreased significantly, with the same aforementioned arrangement of 306 days for SBOTB, 262 days for SBOS, 253 days for SBOTH, 234 days for SBOR, and 230 days for control.

Furthermore, at 30°C, the same previous arrangement of oils, which had a shelf life expectancy of 176 days, 150 days, 146 days, 135 days, and 133 days for control, had a further decline in shelf life expectancy. In the case of 40°C, the shelf life of the evaluated oils decreased dramatically compared to the previous arrangement, with 62 days, 52 days, 51 days, 48 days, and 47 days for control, respectively.

The results revealed that all tested oils withstood temperatures up to 30°C well, and that all oils withstood temperatures ranging between 20-30°C well. In reality, it is still clear that TBHQ is the best single antioxidant for most fats and oils, particularly vegetable oils (Buck and Edwards, 1997). However, in the current investigation, both sage and thyme came in second place in terms of their ability to withstand oxidation under the conditions of the instrument (rabidoxy device) Baydir *et al*., (2021).

Sage, like thyme, contains a number of powerful essential oils that have strong antioxidant properties. Such as Thujone (19.89%), camphor (15.12%), borneol (12.89%), and 1.8-cineole (12.06 percent) are some of the primary essential oils found in sage (Moura-Alves and colleagues, 2020) During the flowering period, (Farhat *et al.*, 2014) revealed the presence of the highest antioxidant components such asrosemarinic acid phenolic diterpenes.

Synthetic antioxidants are well known for their high oxidative stability, however due to the long-term drawbacks of synthetic antioxidants, focus has shifted to natural antioxidants in foods. When sage and thyme were added to soybean oil, they showed promising benefits as natural antioxidants.

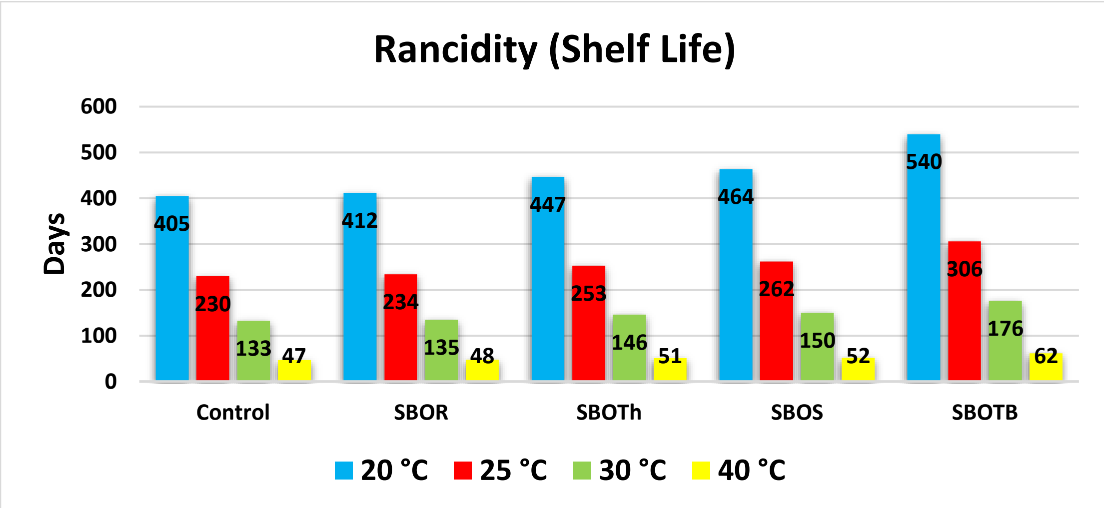

Fig (8) Evaluation of the soybean oil shelf life by rabidoxy device.

SBOC soybean oil sample control, **SBOR** soybean oil + rosemary essential oil, **SBOTH** soybean oil + thyme essential oil, **SBOS** soybean oil + sage essential oil, **SBOTB** Soybean oil + TBHQ.

Sensory evaluation

Sensory evaluation of fried potatoes:

Table (6) shows the sensory evaluation of potatoes fried in different added essential oils used in the present study after 18hrs of heating. As can be seen, panelists scored color of SBOTH, SBOR and SBOS better than control and SBOTB with significant differences between them. Concerning odour, no significant differences were observed between SBOR, SBOTH and SBOTB and were moderately accepted than control and SBOS.

Rosemary and SBOTB were highly scored for taste with no significant differences between them. Panelists showed no significant differences in texture of fried potatoes (crispiness). As for the over all acceptability SBOR, SBOTH and SBOTB gained the highest scores by panelists with no significant differences between them. It was reported by **Chammem *et al.*, (2015)** and **Sayyed *et al.*, (2017)** that the fried potato prepared in oil with rosemary extract had the best crispiness and taste until the fifteenth frying.

Table (6) Sensory evaluation of potato fried in oil with different antioxidants after 18 hr heating

Treatments	Color	Odor	Taste	Texture	Acceptance
SBOC	6.2±1.3^{c}	6.0±0.71^{c}	6.0±1^{c}	6.4±1.14^{ab}	6.4±0.55^{c}
SBOR	6.5±0.89^{b}	6.4±0.71^{a}	7.0±0.84^{a}	6.6±0.89^{a}	6.8±1.1^{a}
SBOTH	7.0±1.24^{a}	6.4±0.5a^{a}	6.5±0.71^{b}	6.6±1.22^{a}	6.7±0.55^{a}
SBOS	6.4±0.55^{b}	6.2±0.45^{b}	6.2±0.84^{c}	6.6±1.14^{a}	6.4±0.71^{b}
SBOTB	6.2±1.72^{c}	6.4±1.21^{a}	7.0±1.51^{a}	6.5±1.05^{a}	6.8±0.98^{a}

SBOC soybean oil sample control, **SBOR** soybean oil + rosemary essential oil, **SBOTH** soybean oil + thyme essential oil, **SBOS** soybean oil + sage essential oil, **SBOTB** Soybean oil + TBHQ.

Sensory evaluation using soybean oil with essential oil at zero time:

Results in table (7) showed that panelists liked all colors of oil with added essential oil at fresh state with no significant differences between them except for rosemary which looked darker in color. They also scored both SBOTH and SBOS highly for odour with no significant differences between them as comared to SBOTB which gained the highest score in this respect. As for the taste, all samples were accepted with no significant differences between them but numerically SBOTH and SBOTB were scored highly. No significant differences (P <0.01) were observed as for texture between essential oils treatments. All treatments were overall accepted by panelists where SBOTH and SBOTB were numerically highly scored

Table (7) Sensory evaluation of soybean oil with added essential oils at zero time

Treatments	Color	Odor	Taste	Texture	Overall acceptance
SBOC	7.0±1.09^{a}	6.0±1.79^{c}	7.0±1.26^{ab}	5.8±1.17^{c}	6.7±0.82^{ab}
SBOR	6.2±0.45^{b}	5.8±0.45^{c}	6.8±0.45^{b}	6.5±0.71^{b}	6.4±0.45^{b}
SBOTH	6.6±0.54^{ab}	6.6±0.55^{b}	7.0±0.71^{ab}	6.6±0.89^{b}	7.4±0.89^{a}
SBOS	6.2±0.45^{a}	6.4±0.55^{b}	6.6±0.55^{b}	6.5±0.45^{b}	6.6±0.55^{ab}
SBOTB	7.0±1^{a}	7.2±1.3^{a}	7.6±0.89^{a}	6.8±0.84^{a}	7.2±0.84^{a}

SBOC soybean oil sample control, **SBOR** soybean oil + rosemary essential oil, **SBOTH** soybean oil + thyme essential oil, **SBOS** soybean oil + sage essential oil, **SBOTB** Soybean oil + TBHQ.

CONCLUSION:

It may by conculated that all essential oil added to soybean oil (rosemary, thyme, sage) showed promising abilities in inhibiting the onset of oxidation in soybean oil under the test conditions of the present study as compared to the strong synthetic antioxidant TBHQ. The shelf life expectancy of the tested oils under the Rabidoxy device conditions can hold well from 20 - 30°C.

REFERNCES

Al-Dalain, S. Y., Al-Fraihat, A.H and Al Kassabeh, E.T. 2011. Effect of aromatic plant essential oils on oxidative stability of sunflower oil during heating and storage. Pakistan journal of nutrition. 10(9): 864-870.

Almoselhy, R.I.M. 2021. Comparative study of vegetable oils oxidative stability using DSC and Rancimat methods. Egypt.J Chem. 64(1): 299-312.

Al-Owaisi, M., Al-Hadiwi, N., & Khan, S. A. 2014. GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of Moringa peregrina (Forssk.) Fiori leaves. Asian Pacific Journal of tropical biomedicine, 4(12): 964-970.

American Oil Chemists' Society Official Method Cd 1-25 (1998) In: Official methods and recommended practices of the American Oils Chemists' Society. AOCS, Champaign

Anwar, F., Chatha, S.A.S and Hussain, A.I. 2007. Assessment of oxidative deterioration of soybean oil at ambient and sunlight storage. Grasas y aceites. 58(4): 390-395.

AOCS.Cd18-90. (2011). Official methods and recommended practices of the American Oil Chemists' Society (AOCS). Champaign, IL: AOCS press.

AOCS.Cd8b-90 (2011). Official methods and recommended practices of the American Oil Chemists' Society (AOCS), Vol. Cd 8b–90. Champaign, IL: AOCS Press.

Baydir, A.T., Soltanbeigi, A., Canlidinic, R.S and Erdogan, M.S. 2021. Determination of chemical properties and antioxidant effect of *salvia officinalis L*. JONAS, 4(1): 95-100

Buck, D. F., & Edwards, M. K. 1997. Antioxidants to prolong shelf-life. Food Technol. Int, 2, 29-33.

Carlsen, M. H., Halvorsen, B. L., Holte, K., Bøhn, S. K., Dragland, S., Sampson, L., Willey, C., Senoo, C., Senoo, H., Umezono, Y., Sanada, C., Barikmo, I., Berhe, N., Willett, W.C., Phillips, K.M., Jr, D.R and Blomhoff, R. 2010. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. Nutrition journal, 9(1): 1-11.

Chammem, N., Saoudi, S., Sifaoui, I., Sifi, S., de Person, M., Abderraba, M., Moussa, M and Hamdi, M. 2015. Improvement of vegetable oils quality in frying conditions by adding rosemary extract. Industrial Crops and Products. 74: 592-599.

Clemente, T.E. and Cahoon, E.B. 2009. Soybean oil: genetic approaches for modification of functionality and total content. Plant physiology. 151(3): 1030-1040.

Cuvelier, M. E., Berset, C., & Richard, H. 1994. Antioxidant constituents in sage (Salvia officinalis). Journal of Agricultural and food chemistry, 42(3): 665-669.

Dobarganes, C., and Márquez-Ruiz, G. 2003. Oxidized fats in foods. Current Opinion in Clinical Nutrition & Metabolic care, 6(2), 157-163.

Duke JA and Ayensu ES 1985. Medicinal Plants of China, Reference Publications, Michigan.

Egan, H., Kirk, R.S & Sawyer, R. 1981. Pearson's chemical analysis of foods, 8th edition, Churchill livingstone.

Embuscado, M.E. 2015. Spices and herbs. Natural sources of antioxidants. J. Function of foods, 18: 811-819.

Farhat, M. B., Chaouch-Hamada, R., Sotomayor, J. A., Landoulsi, A., & Jordán, M. J. 2014. Antioxidant potential of *Salvia officinalis L*. residues as affected by the harvesting time. Industrial Crops and Products, 54: 78-85.

Gamez-Meza, N., Noriega-Rodriguez, J.A., Medina-Juarez, L.A., Ortega-Garcia, J., Cazarez-Casanova, R and Angulo-Guerrero, O. 1999. Antioxidant activity in soybean oil of extracts from Thompson grape bagasse. Journal of the American Oil Chemists' Society. 76(12): 1445-1447.

Goracinova, K., Klisarova, L., Makraduli, L., Fredro-Kumbaradzi, E., Glavas, M., & Mladenovska, K. 2000. Biopharmaceutical characterization of povidone-iodine liposomes. of the Chemists and Technologists of Macedonia, 19,(2): 133-137.

Guenther, E., 1952. The Essential Oils, V, Litton Educational Publishing Inc., USA.

International Union of Pure and Applied Chemistry (IUPAC). 1987. Standard Methods for the Analysis of Oils, Fats and Derivatives, Paquot C and Hautfenne A (Eds.), 7th Revised Version, Blackwell Scientific, London.

Ivanovic, J., Dimitrijevic-Brankovic, S., Misic, D., Ristic, M., & Zizovic, I. 2013. Evaluation and improvement of antioxidant and antibacterial activities of supercritical extracts from clove buds. Journal of Functional Foods, 5(1): 416-423.

Juárez, M.D., Masson, L. and Sammán, N. 2005. Deterioration of partially hydrogenated soybean oil used in deep fat frying of a meat food. Grasas Aceites 56: 53–58.

Longani, M.K. and Davis, R.E. 1980. Effects of lipid oxidation on amino acid in lipid peroxidation on amino acids in lipid peroxide protein interaction lipids. 15: 485-490

Masuda, T and Goldsmith, P.D. 2009. World soybean production: area harvested, yield, and long-term projections. International food and agribusiness management review. 12(1030-2016-82753): 1-20.

Moura-Alves, M., Gouveia, A. R., de Almeida, J. M., Monteiro-Silva, F., Silva, J. A., & Saraiva, C. 2020. Behavior of Listeria monocytogenes in beef Sous vide cooking with *Salvia officinalis L*. essential oil, during storage at different temperatures. LWT, 132: 109896.

Nieto, G. 2020. A review on application and uses of Thymus in the food industry plants, 9:961(doi: 10.3390/plants 9080961.

Nikolić, N.Č., Cakić, S.M., Novaković, S.M., Cvetković, M.D and Stanković, M.Z. 2009. Effect of extraction techniques on yield and composition of soybean oil. Macedonian Journal of Chemistry and Chemical Engineering. 28(2): 173-179.

O'Brien, R. 1993. The use of major fats and oils by the US foodservice industry, which includes all types of commercial establishments as well as institutional food preparers. Inform-champaign-, 4: 913-913.

Official Methods and Recommended Practice of the AOCS, 6th ed.; Firestone, D., Ed.; American Oil Chemists' Society: Champaign, IL, 2009; Official Methods Ac 5-41 and Cj 1-94.

Oyaizu, M. 1986. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. The Japanese journal of nutrition and dietetics, 44(6): 307-315.

Pandey, K. H., Mahalingan, K., Sharma, S., Lamsal, R., & Amritha, V. 2014. Antioxidant properties of various fruits, herbs, spices and vegetables: A review. World Journal of Pharmacy and Pharmaceutical Sciences, 3(2): 1101-1109.

Park, H. 2012. Modifying the fatty acid profile of soybean oil for nutritional and industrial applications. The University of Nebraska-Lincoln.

Peng, Y., Yuan, J., Liu, F and Ye, J. 2005. Determination of active components in rosemary by capillary electrophoresis with electrochemical detection. Journal of pharmaceutical and biomedical analysis. 39(3-4): 431-437.

Petrović, M., Kezić, N., & Bolanča, V. 2010. Optimization of the GC method for routine analysis of the fatty acid profile in several food samples. Food Chemistry, 122(1): 285-291.

Pizzale, L., Bortolomeazzi, R., Vichi, S., Überegger, E., & Conte, L. S. 2002. Antioxidant activity of sage (*Salvia officinalis* and *S fruticosa*) and oregano (*Origanum onites* and *O indercedens*) extracts related to their phenolic compound content. Journal of the Science of Food and Agriculture, 82(14): 1645-1651.

Poiana, M.A., Trasca, T., Moigradean, D and Gaita, C. 2016. Study on thermo-oxidative stability of soybean oil during convective heating at simulated frying temperature. Journal of Agroalimentary Processes and Technologies. 22(2): 108-113.

Prior, R. L., Xu, X., & Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry, 53: 4290–4302.

Proestos, C., Boziaris, I.S., Kapsokefalou, M. and Komaitis, M. 2010. Natural antioxidant constituents from selected aromatic plants and their antimicrobial activity against selected pathogenic microorganisms. Food Technol. Biotechnol. 4:, 524–529.

SAS (1994). SAS user's: Stattistics SAS institute Inc, cary, NC., USA.

Sayyad, R., Jafari, S., & Ghomi, M. 2017. Thermoxidative stability of soybean oil by natural extracted antioxidants from rosemary (*Rosmarinus officinalis L.*). International Journal of Food Properties, 20(2): 436-446

Shahidi, F., & Zhong, Y. 2010. Novel antioxidants in food quality preservation and health promotion. European Journal of Lipid Science and Technology, 112(9): 930-940.

Shehata, M. G., Badr, A. N., Abdel-Razek, A. G., Hassanein, M. M., & Amra, H. A. 2017. Oil-bioactive films as an antifungal application to save post-harvest food crops. Annual Research & Review in Biology, 1-16.

Srinivasan, K. 2014. Antioxidant potential of spices and their active constituents. Critical reviews in food science and nutrition, 54(3): 352-372.

Steenson, D.F and Min, D.B. 2000. Effects of β-carotene and lycopene thermal degradation products on the oxidative stability of soybean oil. Journal of the American Oil Chemists' Society. 77(11): 1153-1160.

Wanasundara, U. N., & Shahidi, F. 1996. Stabilization of seal blubber and menhaden oils with green tea catechins. Journal of the American Oil Chemists' Society, 73(9): 1183-1190.

Wang, W., Wu, N., Zu, Y.G and Fu, Y.J. 2008. Antioxidative activity of *Rosmarinus officinalis L*. essential oil compared to its main components. Food chemistry. 108(3): 1019-1022.

Youdim, K.A.; Deans, S.G.; Finlayson, H.J. 2002. The Antioxidant Properties of Thyme (Thymus zygis L.) Essential Oil: An Inhibitor of Lipid Peroxidation and a Free Radical Scavenger. J. Essent. Oil Res. 14: 210–215.

Zarina, Z., and Tan, S. Y. 2013. Determination of flavonoids in *Citrus grandis* (Pomelo) peels and their inhibition activity on lipid peroxidation in fish tissue. International Food Research Journal, 20(1): 313.

Zhang, Y., Yang, L., Zu, Y., Chen, X., Wang, F., & Liu, F. 2010. Oxidative stability of sunflower oil supplemented with carnosic acid compared with synthetic antioxidants during accelerated storage. Food chemistry. 118(3): 656-662.

Zheng, W., & Wang, S. Y. 2001. Antioxidant activity and phenolic compounds in selected herbs. Journal of Agricultural and Food chemistry, 49(11): 5165-5170.

الملخص العربي

تحسين الثبات التأكسدى الحراري لزيت فول الصويا بأستخدام بعض المستخلصات العشبيه

محمد احمد حسن[١], اميمه السيد شلتوت[٢]، ايمن ابواليزيد[٢]

[١] اويلكس, الشركه الدولية لاستخلاص الزيوت المتعددة. [٢] قسم علوم الاغذية, كليه الزراعه (سابا باشا), جامعة الاسكندرية, الاسكندرية, مصر . ص.ب.٢١٥٣١

يعتبر زيت فول الصويا أحد أهم محاصيل البذور الزيتية في العالم. يستخدم زيت فول الصويا المكرر والمزال الرائحة على نطاق واسع في زيوت القلي وزيوت السلطة والعديد من الأطعمة الأخرى على الرغم من المعاملات التكنولوجية للزيت فإنه لا يزال يحتوي على 84.53٪ من الأحماض الدهنية غير المشبعة مما يجعله عرضة للأكسدة وتطور التزنخ. وبالتالي تهدف الدراسة الحالية إلى الحد من الأكسدة باستخدام مضادات الأكسدة الطبيعية المستخلصة من الأعشاب بدلاً من استخدام مضادات الأكسدة الاصطناعية مثل TBHQ التي قد تسبب مخاطر صحية ومخاوف تتعلق بالسموم. تم تقييم اختبارات الجودة العامة و تقييم الأحماض الدهنية المكونه لزيت فول الصويا المكرر المستخدم في هذه الدراسة. تم استخلاص الروزماري والزعتر والمريمية باستخدام جهاز Clevenger ثم تم قياس الكم المتحصل عليه بعد الاستخلاص. تم تقدير مجموع المركبات الفينوليه و الفلافونويدات في المستخلصات العشبية. تم قياس النشاط المضاد للأكسدة للمستخلصات بطريقتين (DPPH و FRAP) تم دمج المستخلصات العشبية بمعدل 400 جزء في المليون إلى زيت الصويا في وجود عينه ضابطه ومضاد أكسدة اصطناعي TBHQ عند 150 جزء في المليون. تم استخدام اختبار الفرن لتسريع الأكسدة (180 درجة مئوية ± 5 درجة مئوية لمدة 18 ساعة بفاصل 3 ساعات) وتم اختبار النتائج عن طريق قياس قيمة الحموضه ، وقيمة البيروكسيد ، وقيمة اليود ، CD و CT ، وقيمة البارا أنسيدين و قيمه الأكسدة الكلية. تم أيضًا قياس تقدير العمر الافتراضي باستخدام جهاز Rabidoxy من Anton's bar. أظهرت نتائج اختبار الفرن نتائج واعدة للزيوت الأساسية المضافة إلى زيت فول الصويا مقارنةً بالعينه الضابطه و TBHQ حيث أظهر إكليل الجبل تثبيطًا واضحًا للأكسدة في اختبارات الأكسدة الثانوية (قيمة الحموضه والبارا أنسيدين) بينما أظهر الزعتر والمريمية مزيدًا من تثبيط الأكسدة في اختبارات الأكسدة الأولية (قيمة البيروكسيد ، CD، CT وقيمة اليود). أظهر حساب العمر التخزيني باستخدام جهاز Rabidoxy للعينات أفضل النتائج عند 20 درجة مئوية حتى 30 درجة مئوية ولكن جميعها انخفضت عند 40 درجة مئوية تحت الظروف المستخدمة في الجهاز. كانت اختبارات التقييم الحسي بعد 18 ساعة من التسخين مقبولة خاصة لإكليل الجبل والزعتر أما بالنسبة للتقييم الحسي للزيت الذي لم يتعرض للمعاملات الحراريه، كانت جميع العينات مقبولة مع عدم وجود فروق ذات دلالة إحصائية بينها.