

Evaluation Of The Effects Of Processing Parameters Of Roasting On The Antioxidant Activity And Bioactive Molecules Of Seeds Oil Of Sesame (*Sesamum Indicum* .L)

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Abstract: Bioactive compounds, especially antioxidative constituents, play an imperative role in the nutritional and health impact of edible oil. Sesame is one of the world's most important and oldest oilseed crops with a high level content of antioxidant known to human health. The antioxidant, phenolic compounds, tocopherols and lignans are factors responsible for the stability of roasted sesame oil which is highly affected by the conditions of the roasting process. The aim of this study was to Survey of the roasting temperature (150°C) and time effects on antioxidants and phenolic content in sesame oil. The sesame oil was extracted and analyzed for his bioactive compounds. Roasting condition was found to cause an increase in the passage of phenolics, flavonoids, lignans compounds to oil, and the same for the antioxidants activity for the first 2 hours, however, with further roasting time, the antioxidant activity and bioactive compounds were reduced. Those results suggest that a phenolic content and the lignans are the main responsible for the antioxidant potential of sesame.

Keywords: sesame oil, roasting, bioactive, tocopherol, carotenoids, compounds bioactif

I. Introduction

Sesame (*Sesamum indicum*.L) is an important annual oil seed crop being cultivated in the world, as an ingredient in human foods, animal feeds, in different form of use; seed, oil and meal. It has been cultivated for centuries, for its high content in oil and protein, providing an excellent nutritional value and a great resistance to oxidative deterioration of sesame oil and seed, resulting a great a high oxidative stability during storage, processing or heating. The sesame oil known as being high quality used in cooking fats, margarine, salad seasoning. The stability in sesame oils is due to their richness in various endogenous compounds like lignans, tocopherols and phenolic compounds. The lignans have been reported that they could be responsible for high stability and lowering cholesterol levels (Williamson and *al.*, 2008)¹. The tocopherols; a vitamin E compounds have beneficial properties as antiproliferative effects in cancer cells (Gysin and *al.*, 2002)² and preventing hypertension, cancer and can decrease off-flavor compounds formation. Sesame oil prepared from roasted sesame seeds has characteristic odor and taste and longer shelf life which give him higher oxidative stability than other vegetable oils.

Sesame lignans like sesamin, sesamol have shown public interest, with other lignans produced during bleaching like sesaminol, and sesamol formed during roasting, those compounds could improve food quality and antioxidative stability. Antioxidants such as tocopherols, phenolic compounds and lignans are sometimes added to the vegetal oil to decrease oxidation during frying, heating and processing.

Roasting is the most significant step in processing of different seeds, that causes important physical, chemical, structural and sensorial changes, making the oil extracted from roasted sesame seeds was considered more antioxidative and with better sensory and nutritional properties than unroasted sesame oil (Fukuda and *al.*, 1986)³. The roasting process could promote more flavor, desired color and increasing the palatability, and to improve the processing efficiency of subsequent treatment. But those beneficial effects of roasting are influenced by the roasting conditions. So, in order to make good quality, the optimum roasting conditions should be established, optimal conditions in temperatures and times are required and also dependent on different factors; degree of roasting, roaster type, type of the vegetal material, those factors have to be controlled because roasting causes desirable and undesirable changes in physical, chemical and nutritional properties of the oil. One of the main desired outcomes of roasting process is the increase in antioxidant activity that occurs mainly due to

the formation of Maillard reaction products. It was reported that sesame seeds were roasted at 90-100°C for sesame paste production. It was also advised hot air roasting at 130 °C for 1 hour (El-Adawy and *al.*, 2000)⁴. On the other hand, Ozcan and *al.*, (1994)⁵ reported that sesame should be roasted at 100–150 °C for 2.5–3 h for the production of sesame paste. Since the roasting affects the product quality, sensorial quality and the color; the control of roasting process is significant. So, different cooking practices can affect the products for example the acrylamide content of foods. This Acrylamide content can be correlated to food browning. Those compounds are formed in the Maillard reaction by the reaction of the free asparagines and reactive carbonyls at temperatures above 120°C (Stadler and *al.*, 2000)⁶.

The control of roasting operation is very important to prevent the apparition of many toxic compounds and off-flavor. The aim of this study was to establish the optimum level of roasting conditions with a good quality of sesame oil because it was the one of the main problems for the oil sesame producers. The purpose of the present study was to elucidate the relationship between roasting temperature and time on the antioxidant activity, phenolic content, lignans, tocopherols, chlorophylls and carotenoids of extract from a sample of sesame oil roasted and extracted.

II. Materials and Methods

Sesame oil preparation

Sesame seeds were roasted at the temperature of 150°C with the duration of 6 hours. During roasting process samples were taken at different time intervals (30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360 min), and they were immediately equilibrated to room temperature for prevent further heating, Therefore 13 roasting conditions were used in this study, roasted seeds were packed in polyethylene plastics bags and stored for analysis. Oil from roasted sesame seeds was extracted using a soxhlet apparatus with hexane for 8hour per sample. Control sesame oil was prepared from sesame seed roasted at the same conditions. All the samples were prepared triplicates.

Methanolic extract of sesame oil

One gram of oil sample and 1ml of 80% methanol were mixed in an eppendorf tube and agitated with a vortex mixer for 2 min. Samples were left in a separating funnel 1 hour and the upper methanolic phase was taken. One millilitre Fresh methanol (80%) was added and the extraction was repeated three times, upper phases were combined (Durmaz and *al.*, 2011)⁷. The final methanolic solution was used for total antioxidant capacity, total phenolic and flavonoid contents analyses.

Analysis of total antioxidant activity

Antioxidant potential of the oil extract of the roasted sesame was measured using DPPH (1,diphenyl-2-picryl hydrazyl) method(Leitao and *al.*,2002)⁸. Briefly, 1.5 ml of diluted DPPH in methanol was added to 10 µl of the oil extract. A control without a sample was used. The solution was stirred and maintained in the dark for 45 min, the absorbance was measured at 515nm. The antioxidant activity was calculated using the following formula:

The antioxidant activity (%) = ((Absorbance sample -Absorbance control) / absorbance control) * 100.

Determination of total phenolic content

Total phenolic content was determined using Folin-Ciocalteu colorimetric method and expressed as mg of gallic acid equivalents (GAE) per gram extract(Singleton and *al.*, 1965)⁹ 0.5 ml of the oil extracts were added to 2.5ml of Folin-Ciocalteu diluted 10 times and 2 ml of an aqueous solution of Na₂CO₃ (75g / l). The solution was stirred and heated 5min on 50°C. The absorbance was measured at 760 nm.

Determination of total flavonoid content

The flavonoid content was determined; 1ml of the extract methanolic was added to 1ml of aluminium trichlorid ALCL₃ (2%). After 15 min of incubation. The absorbance was measured at 430 nm and the results were expressed an mg quercetin equivalents per mg extract (Ordon and *al.*, 2006)¹⁰.

Determination of color development

As an index of color development, the absorbance at 240 nm of 5.0% (w/v) solution of oil in chloroform was determined with a spectrophotometer (UV-900, Jasco) (Yochida and *al.*, 1997)¹¹.

Determination of lignans contents

The lignans contents were determined by the method of ⁽¹²⁾ by dissolving 0.01g of oil samples in 10 ml of hexane-chloroform mixture (7:3.v/v) and the absorbance at 288 nm was deduced. The lignans content was calculated by using the formula:

% lignans as sesamol = [(A/W)*(100/230.1)]
% lignans as sesamin = [(A/W)*(100/231.1)] where,
A: absorbance of the sample
W/weight of the sample in gram/100ml
230.1 E^{1%} 1cm for sesamol
231.1 E^{1%} 1cm for sesamin.

Determination of the chlorophyll and carotenoid contents

Chlorophylls and carotenoids contents were determined (Mosquera and *al.*,1991)¹³. For the procedure, 0.75 g of oil was weighed exactly, dissolved in cyclohexane and taken to a volume of 2.5ml. The chlorophyll and carotenoid fractions were determined at 670 nm and 470 nm. Carotenoid content was calculated using Equation namely: "Carotenoid content=(A470×25×10000)/(E0×7.5)" in which, A470: Absorption maximum at 470 nm; E0: Specific extinction (2000).

Tocopherols analysis

100 mg of sesame oil was dissolved in 5.0 ml of Absolut ethanol. After mixing the samples, they were filtered with a 0.45µm Nylon membrane and injected into an HPLC column. HPLC analysis of tocopherols in sesame oil was performed using the external standard method by Agilent 1100 high-performance liquid chromatography equipped with C18 column (4.6×150 mm, 5µm), a mobile phase consisting of 50% acetonitrile (A) and 50% methanol (B) was used with a flow rate of 1.0 ml/min. The column compartment was controlled at 25°C and the injection volume was 20µl. The eluate was detected using a fluorescence detector set at an emission wavelength of 325 nm with an excitation wavelength at 295 nm (Gliszczyn'ska-S'wigło and *al.*,2004)¹⁴.

Statistical analysis

Statistical analyses were conducted using SPSS (Statistical Program for Social sciences) version 20.0 (Version 20, SPSS Inc., Chicago, IL, USA). All analyses were performed in triplicate and data reported as means ± standard deviation (SD)

III. Result and Discussion

We did evaluate phenolic, lignans, tocopherols content and antioxidant activity of sesame oil from roasted and unroasted sesame seeds because those compounds are believed to be bioavailable and bioactive. The results shows that the antioxidant activity increased significantly as the roasting temperature was fixed at 150°C during the first 90min and then decreased by roasting at 150°C starting from 120 min to 360min. So, we can say the highest activity and content were achieved by roasting at 150°C for 90 min. For the control (sample of sesame oil without roasting seeds), the antioxidant activity was 60, 98%, then for the 30, 60, 90 min of roasting; this antioxidant activity increased to became 61.20, 61.50 and 61.50% respectively which can be related to the natural antioxidant present on the sesame or the phenolic compounds, the antioxidant activity begins to decrease until reaching 45.98% in 360 min of roasting. But even so, we can see that the oil of sesame seeds remind stable for 2 hours which it due to his highly resistant to oxidative deterioration as compared to other edible oils (Figure1). Its remarkable stability is due to the presence of a large quantity of endogenous antioxidants and phenolic compounds, comprised of flavonoids, sesamin, sesamol and tocopherols. And even though it contains nearly 85% unsaturated fatty acids; the oxidative stability of sesame oil is superior to that of other vegetable oils and compared to them sesame oil showed higher oxidative stability (Sadeghi and *al.*, 2009)¹⁵.

The relatively greater oxidative stability of oils from roasted sesame may be resulting from the formation of some new antioxidants. It was found that the sesame oil prepared at 150-200°C roasting temperature had the best flavor and a longer shelf-life when compared with the others. It was believed that the storage stability of unroasted oil in low, but the roasting process increased stability of sesame oil. His oil extracted from roasted sesame seeds at 180-200°C was considered much more antioxidative than unroasted purified sesame oil. It was also believed that the oxidative stabilization of vegetable oil achieved by the addition of sesame extracts could be attributed to the presence of natural antioxidant which increase during roasting. Furthermore, the enhanced oxidative stability observed in the case of sesame seed extracts and oil might be correlated with their high content in phenolic compounds. In general, it is known that DPPH radical scavenging activity and the level of phenolic compounds are correlated.

Level of total phenolics was determined by the Folin-Ciocalteu method. Those bioactive compounds are distributed in plants with biological and functional properties that are important in terms of food nutritional quality and human health. The phenolic contents have a role of protection against biotic (pathogen attack) and abiotic factors (UV, drought and salt stress). The thermal treatment applied to foods or oil by heating, bleaching or roasting cause's evaporation of intracellular water, triggering chemical reactions that can change the

lignocellulosic structure and promotes protein denaturation, which could give a greater availability of plant phenolic compounds in the matrix. However, data is lacking in studying the contribution of roasted sesames to dietary polyphenol intake since most commercial sesame are roasted when using gastronomically used and the roasting process should be controlled in order to provide the best flavor and the high nutritional quality.

In this study, the amount of total phenolic content and flavonoids increased significantly as the roasting time until 90 min (Figure2) and (Figure3). For the control, the total phenol content and flavonoids were 86, 70 and 0,092 mg/kg. Those molecules bioactives increased to achieve 87.4; 87.45; 87.55 mg gallic acid/kg of oil and 0.096; 0.096; 0.095, 0.095 mg quercetin/kg of oil in the times 30; 60; 90; 120 respectively for both phenolic and flavonoids content. Those molecules begin to decrease until reaching 73.98 and 0.050 mg/kg in 360 min of roasting. In fact, it was reported that the presence of phenolic compounds increased if it is extracted from roasted seeds, but in this study, roasting for a long time (more than 2hours) caused a reduction of the phenolic compounds, these results suggest that phenolic content present in the sesame oil could be thermo labile. Many authors have demonstrated a positive correlation between total phenolic content and the antioxidant activity of fruits, vegetables and oils (Wang and *al.*,2009)¹⁶. The presence of natural antioxidant as phenolic content reflects presence of naturally occurring neo-formed antioxidant constituents in oils obtained from roasting, this process caused a clear increase in this activity for the first 2 hours that was measured by DPPH assay, and there was a decrease with the extension of the roasting, a similar trend was observed for total phenolic and flavonoids content that increased by 2 hours of roasting compared to unroasted sample. Further roasting caused a fluctuation and no more increase was observed. This increase is related to the relatively polar compounds in oil that were accumulated during roasting. Phenolic compounds were reported to pass into the oil phase better if the oil was obtained from roasted seeds which are probably caused by the release of phenolic compounds from bound structures or chemical alteration of phenolics at higher temperatures.

The correlation between the concentrations of total phenolic, flavonoids content and the antioxidant activity performed by Pearson's test showed a high and significant positive correlation at the level 0.01 ($R=0.950$) for polyphenols and $R=0.895$ for flavonoids) for phenolic compounds in all the samples with different time, suggesting a strong involvement of phenolic compounds in the antioxidant activity measured by DPPH methodology. This activity was found to be relative to the phenolic compounds; the increase of those compounds resulted the increase of the antioxidant activity for 120 min, while the elongation of roasting reduced the activity and the phenolic compounds. This is due to the fact that the chemistry behind these methods is based on the same redox properties. Within the seeds, increases in moisture during the accelerated ageing could activate lipoxygenase, leading to formation of lipid peroxidation products which might then interact with the skin (Buranasompob A., and al 2007)¹⁷. Further studies are warranted to elucidate the mechanisms underlying the increase of polyphenols content in almond skins during storage.

Color is one of the most important quality attributes evaluated by consumers, producers and distributors. The chlorophyll and the carotenoids are both responsible for the color of the oil, but the pigment concentrations are influenced by a number of factors like geographic origin, degree of ripeness, storage conditions and processing method, those pigments are widely affected by heat treatment which occur degradation and a change of color.

The color development of sesame oil prepared at 150°C and different roasting time is shown in Figure 4. With increasing the roasting time, browning substances were developed, resulting in significant ($P<0.05$) increase of the absorbance at 420 nm. The color of oils from roasting sesame seed changed gradually from light-yellow (for the unroasted sample with the absorbance 0.0932 nm and 0.1013 nm for the sample from 30 min of roasting) to brown with the absorbance 0.243, 0.265, 0.276 nm at 120,150,180 min of roasting, and finally to deep brown at 330 and 360 min of roasting with 0.592 and 0.618 nm respectively. Therefore, the color formation in the oil was influenced by roasting high temperature and the long-time of roasting. The formation of browning substances results from Maillard –reactions between reducing sugars and free amino acids and also the formation of acrylamids. The color formation in sesame oil during heating processes could be attributed to both non-enzymatic browning and phospholipids degradation during roasting (Husain and *al.*, 1986). . The non-enzymatic browning is favored by heat treatment and includes a wide number of reactions such as Maillard reaction, caramilisation and chemical oxidation of phenols. For instance, in the case of Maillard reaction, the sesame seed contains the sugars and amine groups as found in protein molecules, required for Maillard reaction. Thus, the color formation during heat treatment is partly due to the formation of colored MRPs, which is correspond to compounds with a low-molecular-weight and melanoidins with high-molecular-weight (Yoshida and *al.*,1997)¹⁹. Previous studies have reported that an increase in the roasting temperature of seeds, such as sesame seed resulted in significant increase in color of oils, which is identical and consistent with our results.

Chlorophyll and carotenoid pigments are highly appreciated as functional components both for its coloring properties and its health benefits for the human consumption. Carotenoids, besides their participation in coloring of fruit, vegetables and oils, are bioactive compounds which have provitamin A function and antioxidant activity and prevent age-related macular degeneration and cataract formation, and also it has been

demonstrated that the chlorophyll compounds, in addition to its function as green coloring, exhibit a series of biological properties such as antioxidant activity. The chlorophyll and carotenoid concentrations were measured in sesame oil from raw and roasted sesame seeds; Roasting treatment also an increase in green pigment and particularly in chlorophyll content for the first 90 min with the values of 0.19 $\mu\text{g/g}$ compared with the unroasted sample with the value 0.17 $\mu\text{g/g}$ showed that roasting treatment produced an increase in the oil chlorophyll pigment and then start to decrease in 120 min with the value 0.16 $\mu\text{g/g}$ to rush in the 360 min 0.065 $\mu\text{g/g}$ (Figure 5), it was reported that roasting causes an increase in the dark, red and yellow units of color. The color formation in sesame oil during heat treatment is probably due to non-enzymatic browning (Maillard reaction) that occurs during roasting. Furthermore the roasted sesame oil was darker and yellower than raw sesame. This color would be due probably to the presence of carotenoids, coloring substances currently used in the industries of fatty corpses.

For the carotenoids, traditional thermal treatments could induce their degradation, in this study, the carotenoids concentration increase with the increase in the time of roasting. Those compounds remained after roasting; they showed a significant increase for 150 min at 150°C of roasting. No degradation was observed in the carotenoid content, on the contrary, the carotenoid increased significantly with the value 3.525 $\mu\text{g/g}$ compared to the unroasted sample with the value 2.75 $\mu\text{g/g}$, this rise could be explained by the fact that the application pressure and temperature lead to softening of the plant tissue and denaturation of proteins that could help to release carotenoids. After the 180 min, the carotenoids remind inalterable compared to raw products and show a slight decrease until arrive to 2.05 $\mu\text{g/g}$ in the 360 min of roasting time (Figure 6). The same results were reported for spinach and other vegetables which show a great stability of carotenoids during process of high treatment (Sanchez and *al.*, 2014)²⁰. Compared to carotenoids, the chlorophylls shows a degradation and decrease with further roasting time which cause's deterioration of green color, which produce an intense color change to brown.

Lignans are a group of natural compounds which are defined as an oxidative coupling product of β -hydroxy phenyl propane. Sesame oil lignans are reported to have unique bioactive, functional, physiological and nutritional properties. Those compounds have a great antioxidative activity which is due to the antioxidative lignin-type compound, sesamol which is formed from sesamol during the roasting process, it was also believed that the oxidative stabilization of vegetable oil achieved by the addition of sesame extracts.

In the case of lignans, roasting causes increases only for the first 2 hours, and began to decrease in both sesamin and sesamol compounds. The decrease of sesamol was greater than sesamin, it could be due to the roasting temperature which decompose sesamol to sesamol. It was reported that sesamol is detected as trace in unroasted sesame oil contrary to roasted oil where a good increase in this content is observed.

Sesamin concentration was increased for the first 60 min until the value 62.69 mg/kg compared to unroasted sample with the value of 57.27 mg/kg, and start to decrease from 90 min to rush 35,34mg/kg on 360 min of roasting. Sesamol is a potent phenolic antioxidant. sesamol was detected in low amounts in raw sesame oil with the value of 57.52mg/kg compared to roasted sesame oil, the sesamol has increase significantly which is due to sesamoline hydrolysis during the thermal oxidation resulting an increase in sesamol after roasting with 71.61 mg/kg in 90 min, 69.47mg/kg in 180 min (Figure 7) due to the conversion from sesamol which decreased the autoxidation of oil. It was reported that this component has an important preventive effect against the thermal decomposition of tocopherol when added individually to other oil to ensure stability (Kajimoto and *al.*, 1992)²¹. The correlation between the concentrations of sesamin, sesamol and the antioxidant activity performed by Pearson's test showed an also a high and significant positive correlation at the level 0.01 ($R=0.875$) for sesamin and $R=0.762$ for sesamol) for lignans compounds in all the samples during the first two hours. The high oxidative stability of roasted oil could be attributed to endogenous antioxidants (lignans) together with tocopherols and phenolic compounds, and specially sesamol. Also the antioxidant factors responsible for the stability of roasted sesame oil seemed to be highly affected by the conditions of the roasting process. It was also reported that phenolic extract from roasted sesame oil possess antioxidant properties and could be used as alternative natural antioxidants with wide food applications.

Tocopherols present a very important compound compared to lignans for the stability of sesame seeds and oil during roasting processing. This compound is used to decrease lipid oxidation by donating hydrogen to lipid peroxy radicals. The results shows tocopherols levels were increased during the first 120 min until 289 $\mu\text{g/g}$ compared to unroasted sample with the value 211 $\mu\text{g/g}$, and start to decrease until 145 $\mu\text{g/g}$ in 360 min which could be due to the high degree of oxidation after a long time of roasting proving that the processing in sesame oil may affect the levels of tocopherol in oils. A high correlation existed between tocopherol and antioxidant activity for each cultivars in all the extracts studied with $p<0.05$, the coefficient of correlation was 0.9013. No single compound can be considered responsible for this stability, a combination of a number of minor constituents such as polyphenols, flavonoids, tocopherols, lignans and carotenoids could have a synergistic role in increasing the oxidation stability.

IV. Conclusion

The chemical composition and oxidative stability of sesame oil prepared from roasted sesame seed at 150°C and different times were evaluated and compared to unroasted sesame oil. Roasting processing changed the polyphenol, lignans, tocopherols, chlorophyll, carotenoids, color and antioxidant content significantly, and to obtain the highest antioxidant activity and high values of bioactive compounds from sesame oil, sesame should be roasted for 2 hours at 150°C. From ancient time, sesame oil has been considered valuable because of its medical effects. Some valuable components in sesame contribute to a nutritional and functional food for humans. The results revealed also that raw sesame and roasted sesame oil were a rich source of many important nutrients that appear to have positive effects on human health. The roasting process suggested that sesame oil could be utilized as a potential source of edible oils for human consumption. For further results, the roasting conditions should be optimized using a statistical technique like response surface methodology which is based on changes in the physicochemical quality indicators during roasting.

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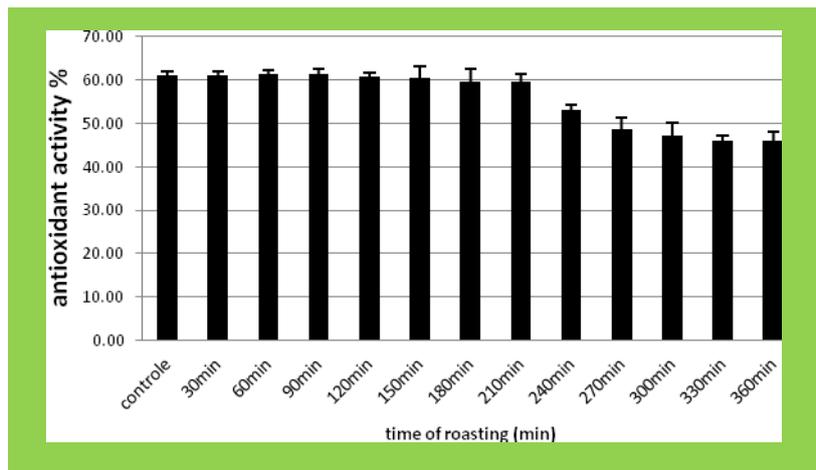


Figure 1. Effects of roasting temperature and time on the antioxidative activity

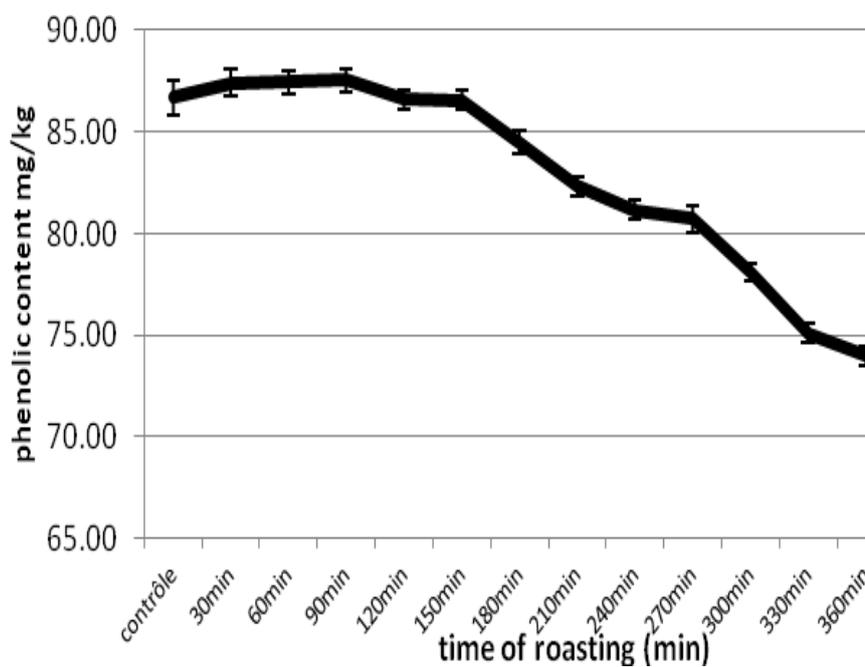


Figure 2. Effects of roasting temperature and time on the phenolic content (mg quercetin/kg of oil)

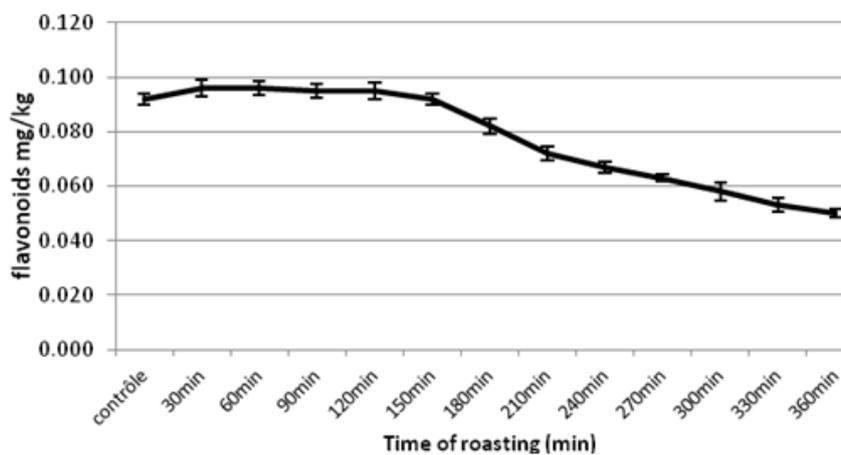


Figure 3. Effects of roasting temperature and time on the flavonoids content (mg quercetin/kg of oil)

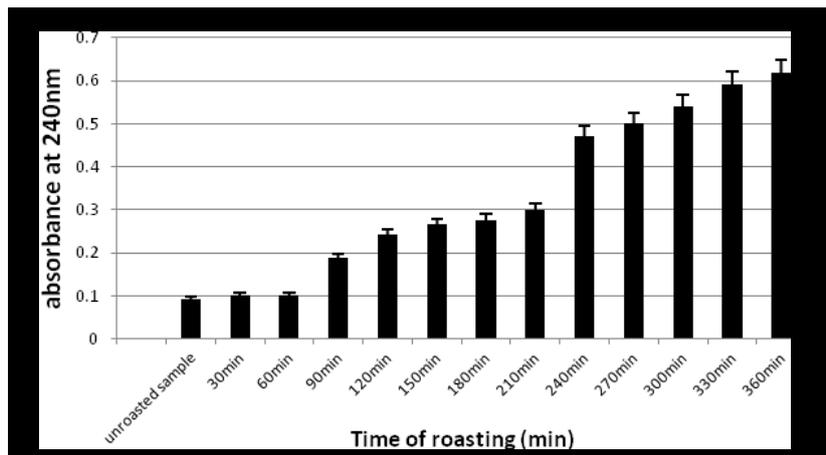


Figure 4. Changes in absorbance (color) of sesame oil prepared from roasted sesame seeds (Changement de couleur de l'huile de sesame préparé par les graines de sesame torrifiées)

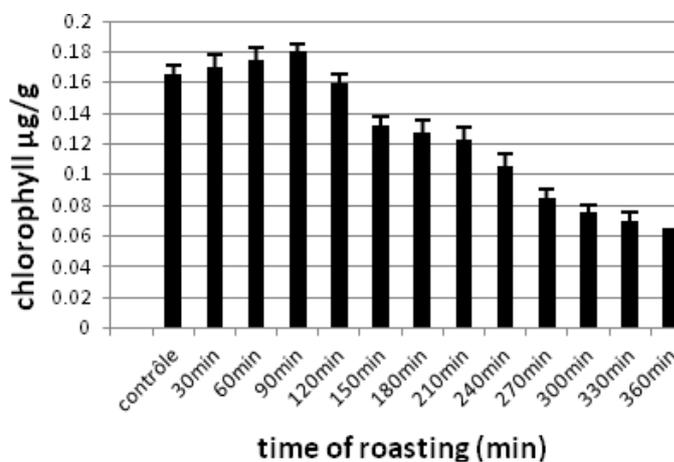


Figure 5. Effects of roasting temperature and time on the on the chlorophyll content (µg/g of oil) (Effets de température et de temps de torrification la teneur des chlorophylles (µg/g of oil))

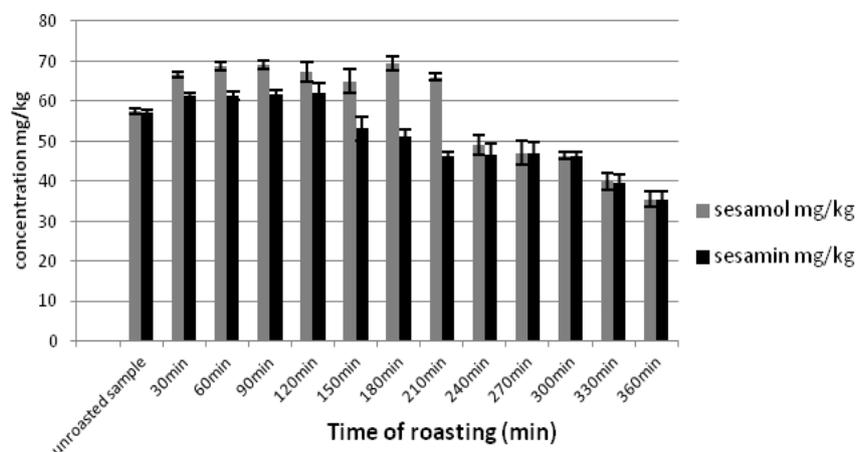


Figure 6. Effects of roasting temperature and time carotenoids content (µg /g of oil) (Effets de température et de temps de torrification sur la teneur des caroténoïdes (µg /g of oil))

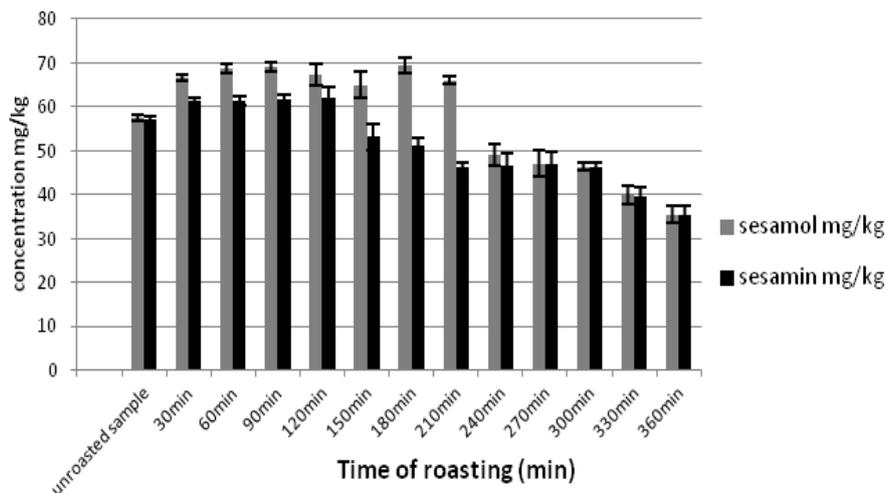


Figure 7. Changes in concentrations of sesamol and sesamin of sesame oil prepared from roasted sesame seeds

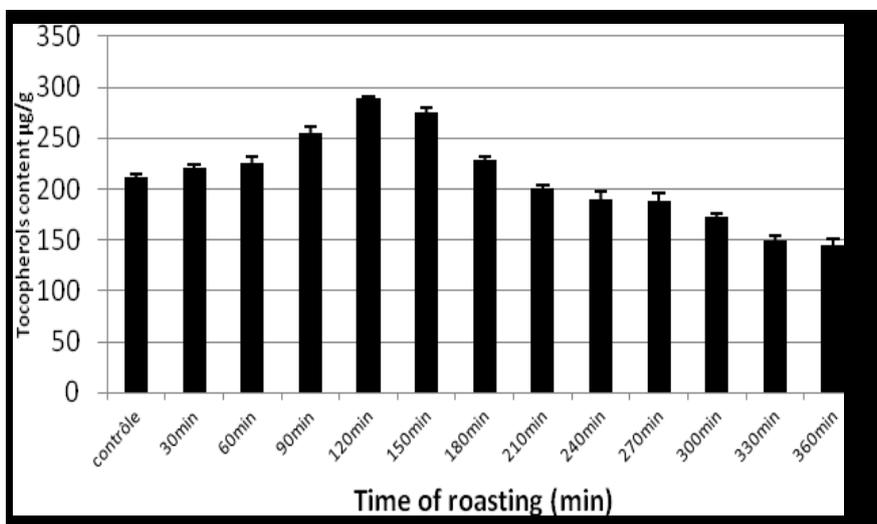


Figure 8. Changes in concentrations of tocopherols (µg/g) of sesame oil prepared from roasted sesame seeds