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# Antioxidant effect of natural rosemary on the oxidation of mid-oleic sunflower frying oil on chicken wings

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**Abstract**

Fried foods are hugely popular and very much a part of our modern cuisine. Guarding against oxidation is crucial, due to the absorption of oil during the frying process. This paper presents how, the degree of resistance of sunflower oil was evaluated during repeated fries of chicken wings by adding the natural antioxidant extracted from rosemary. It was compared to tocopherol and to a control sample. A preliminary test of accelerated aging demonstrated the effectiveness of rosemary extract by using the Rapidoxy technique on sunflower oil. This proved that the adequate dose of this antioxidant should be 600 ppm. Many physicochemical transformations were inflicted on the oil during its oxidation. The kinetics monitoring of this phenomenon was determined by measuring the total polar materials (TPM), free fatty acids (FFA), and the peroxide index (PI). The obtained results showed that all the parameters analysed tended to increase over time. However, the effect of the rosemary antioxidant was positive on the stability of the oil and the slowing down of its oxidation. Moreover, its efficiency was higher than tocopherol. Rosemary antioxidants could be a safer alternative to synthetic antioxidants which represent toxicological risks, such as buthylated hydroxyanisole BHA, buthylated hydroxytoluene BHT, and tert-butylhydroquinone TBHQ.

**Keywords:** oxidation; rosemary; antioxidant; TPM; FFA.

**Practical Application:** Rosemary extract is an excellent antioxidant that protects frying oils against oxidation.

## 1 Introduction

Deep frying with vegetable oils is one of the most widespread and oldest cooking techniques used in the preparation and production of food in both the cooking industry and the household environment (Wu et al., 2019). Sunflower oil is one of the most preferred vegetable oils from an economic and a nutritional perspective (Romano et al., 2021). It is commonly used for frying due to its composition rich in essential polyunsaturated fatty acids (FA) that have more than one double bond, such as oleic, linoleic acid, and alpha-linolenic acids (Romano et al., 2021; Alves et al., 2022). However, heating vegetable oils at high temperatures (> 150 °C) usually leads to a series of chemical reactions such as lipid oxidation and polymerization causing the formation of many hazardous substances (Ganesan et al., 2019). These substances are hydroperoxides, free radicals, acrylamide and heterocyclic compounds (Urbančič et al., 2014; Erickson et al., 2022). Furthermore, some of these compounds are potentially toxic (neurotoxic, genotoxic) carcinogenic, and mutagenic which may contribute to the emergence of serious health issues (Kalyanaraman, 2013; Ganesan et al., 2019). In addition to the alteration of the nutritional and organoleptic quality of the fried foods rancidity is triggered (Aladedunye & Przybylski, 2009; Olmedo et al., 2018). Thus, synthetic antioxidants including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butyl hydroquinone (TBHQ) are those that are most frequently added in order to extend the period of use of frying oil (Cömert & Gökmen, 2018). Nevertheless, because of their harmful effects, some of them have been banned or used with specific legal limits recommended in Europe and the USA (Blasi et al., 2018; Mikołajczak et al., 2020). Currently, natural alternatives are now the tendency to overcome this shortcoming. Therefore, several studies are focused on natural antioxidants extracted from natural plants to improve foods quality (Zhang et al., 2017; Al-Hijazeen, 2022). Among these plant extracts, rosemary is a popular natural antioxidant that is widely utilized in the food industry (Wu et al., 2019). Its antioxidant effectiveness in different frying vegetable oils has been proven by many scientists to be higher than that of synthetic antioxidants (Urbančič et al., 2014; Nieto et al., 2018; Erickson et al., 2022). This antioxidant capacity was mainly due to the presence of rosmarinic acid, carnosic acid, carnosol, and rosmanol. They can be selectively extracted by adapting the solvent polarity, and are able to avoid and scavenge free radicals and interrupt the peroxidation reaction (Loussouarn et al., 2017; Marchev et al., 2021). For these reasons our research focused on how to slow oxidation and to limit the propagation of free radicals in frying oils by using a natural antioxidant extracted from rosemary leaves.

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## 2 Materials and methods

### 2.1 Antioxidant extraction

The extraction of the antioxidant from rosemary was performed according to the method of Rosemary leaves were collected from natural populations in the Guercif region of Morocco and were extracted using hexane. This extract was then filtered, concentrated, and stirred with a sodium hydroxide solution. After separation of the aqueous and organic phases, the solution was acidified with concentrated sulfuric acid, then extracted with dichloromethane to recover the antioxidant material. Furthermore, it was added to an ethanolic extract, filtered from rosemary residue. The mixture was concentrated in a rotavapor and finally dried in a vacuum oven to prevent the deterioration of the antioxidants present. The extract obtained was analysed by UHPLC Acquity H-class (Waters brand), with pulsed amperometric detection PAD over a measurement range of 284 nm, using analytical standards of carnosic acid and carnosol. The powder was diluted in vegetable sunflower oil to facilitate its incorporation into the frying oils.

### 2.2 Frying oil

The frying oil used is a mid-oleic sunflower oil without additives, supplied by the American company "Columbus Vegetable Oils", which contains 58% of oleic acid and 31% of linoleic acid.

### 2.3 Frying oil oxidation kinetics

Before starting the successive frying cycles, the rosemary extract was tested with a Rapidoxy (Anton Paar, Austria brand), an instrument that measures oxidation stability under accelerated conditions (Kerkel et al., 2021), conforming to standard methods ASTM D7525 (American Society for Testing and Materials, 2019) and EN 16091 (European Committee for Standardization, 2012). To verify the rosemary extract's effectiveness, sunflower oil was treated with this extract compared to tocopherol using two doses 600 and 800 ppm of each. They were compared to the control oil without treatment with Rapidoxy at 175 °C. After having determined the adequate dose of rosemary antioxidant, it was added to the sunflower oil, heated to 175 °C, and used to perform 5 successive fries per hour of chicken wings. Each cooking requiring 10 minutes, with a chicken wings/oil ratio of 1/10 weight/weight until total oxidation of the oil. Sunflower oil treated with tocopherol and control oil without treatment were made under the same conditions. As the frying process progressed, the quality of the oil was monitored to follow the evolution of oxidation products by measuring the following parameters: polar compounds, free fatty acids, and peroxides.

### 2.4 Preliminary accelerated oxidation test: Rapidoxy

The measuring principle is based on an artificial acceleration of the oil oxidation process by using high temperature and excess pure oxygen (European Committee for Standardization, 2012; American Society for Testing and Materials, 2019; Kerkel et al., 2021). When oxygen is consumed by the sample, the pressure decreases accordingly. The test aim was to validate the performance of the rosemary antioxidant extract against the oxidation of the oil and to compare it with that treated with tocopherol and the control. The appropriate antioxidant dose for mid-oleic sunflower oil was also tested in Rapidoxy at 175 °C, using the same doses described above.

### 2.5 Total Polar Material (TPM)

The total polar materials (TPM) represent all of the polar degradation products resulting from the frying process, including those from fried foods. In oil, they reflect the rate of deterioration and dissociation of its triglycerides. The analysis was carried out by a probe tester (Testo 270): Deep-frying Oil Tester (Testo, Germany) according to the manufacturer's instructions. It can measure the dielectric constant variations in the oil, strongly correlated with the concentration of TPM, with an accuracy of ± 2% of TPM (Chen et al., 2013). According to French regulations, the maximum percentage of polar compounds allowed is 25%. Beyond this value, the oil is considered unfit for consumption (Firestone, 2007).

### 2.6 Free Fatty Acids (FFA)

Free fatty acids (FFA), responsible for the acidity of the oil are formed by thermal hydrolysis where the triglyceride molecule reacts with a water molecule to give an FFA and a diacylglycerol. This reaction occurs during frying due to the presence of water released from fried foods and the high temperatures used during this process (Kalapathy & Proctor, 2000). FFA were used to characterize the degree of deterioration of frying oils. They are measured according to the official method of the American Oil Chemists Society (AOCS Ca 5a-40, 2017) by neutralizing free fatty acids with 0.1N of sodium hydroxide in the presence of ethanolic phenolphthalein as a colour indicator. The maximum recommended value of FFA in a frying oil is 0.9% and ideally ≤ 0.6% (Freire et al., 2013).

### 2.7 Peroxide Index (PI)

The peroxide index (PI) is used to evaluate the degree of primary oxidation in the frying oil. It quantifies the number of active oxygen molecules in the organic chains of fat that lead to the formation of hydroperoxides. These are responsible for the peroxidation of unsaturated fatty acids and gradually decompose into secondary oxidation products such as aldehydes and hydrocarbons responsible for the rancid flavour and for the bad taste (Ruíz et al., 2001). The higher the index, the more fat material is oxidized (Park & Kim, 2016). The PI was determined according to the iodometric method (International Organization for Standardization, 2017) which requires titration with a sodium thiosulfate solution 0.01N, of the iodine molecules released following the oxidation of iodides by the hydroperoxides of the solubilized oil in an acetic acid/isooctane mixture.

### 2.8 Statistics

All the analyses were performed in triplicate and the results were stated as mean values. The statistical data was processed using the ANOVA function (Fisher's test) in Stat graphics centurion 16 software, to compare the means tests of the two treatments and the control for each duration of frying at different times during t0, 3, 6 and 9 hours. The difference was statistically significant at $p < 0.05$. Then, a complementary Tukey test was made in order to compare the contrasts between two means of variables. Values with identical letters can be considered statistically identical.

## 3 Results and discussion

### *3.1 Physico-chemical analyses of the extract*

The chromatogram obtained by UHPLC (Figure 1) showed that the carnosic acid peak was the most abundant with a content of 42% followed by Carnosol at 12%. A study conducted by Oliveira et al. (2018) used UHPLC as an analytical tool to quantify phenolic compounds in aromatic plants or their extracts. Velamuri et al. (2020) also found carnosic acid and carnosol in abundance as the main diterpenoids found in rosemary analysed by UHPLC.

### *3.2 Preliminary accelerated oxidation test: RapidOxy*

Figure 2 shows the pressure drop that indicates the total oxidation of the oil which starts from 786 seconds for the control. The values of these pressure drops are reported in Table 1. The obtained results show that the control and the treated oil with tocopherol oxidize faster than the one treated with the rosemary antioxidant at 600 and 800 ppm. The rosemary antioxidant at a concentration of 600 ppm was able to last longer than the other concentration before its pressure dropped which represents the total oxidation of the oil. Thus, this concentration allows us to deduce that this is the best solution for consecutive frying. Therefore, all tests were performed with 600 ppm. These results are in agreement with the study of Guitard et al. (2016) who investigated the autoxidation of linseed oil via RapidOxy in the presence of the different phenols including carnosic acid, carnosol, and tocopherol. They also confirmed that these rosemary extracts are the best natural alternatives to α-tocopherol and synthetic phenolic antioxidants (Guitard et al., 2016). In addition, a similar study used RapidOxy to monitor the accelerated oxidative stability of sunflower oil, which decreased with each refining step since the crude oil was more stable than the refined one, indicating that it lost antioxidant molecules during this process (Rhazi et al., 2022). Bano et al. (2022) demonstrated that date varieties at various maturity stages had an important antioxidant activity (Bano et al., 2022). Therefore, it is necessary to add antioxidants, preferably natural ones, to the refined oils used for frying.

**Table 1**. Pressure drop of the oil samples demonstrating their total oxidation.

| Sample | Control | ROA 600 ppm | ROA 800 ppm | Toco 600 ppm | Toco 800 ppm |
|---|---|---|---|---|---|
| Pressure drop time (Seconds) | 786 | 904 | 875 | 833 | 826 |

### *3.3 Total Polar Material (TPM)*

Figure 3 shows TPM tracking during the frying of chicken wings in the control, and in treated oil with tocopherol or rosemary antioxidant. The results show a significant increase in polar compounds in the control sunflower oil which reaches the maximum authorized level of 25% after 25 frying (5 hours). However, this level is reached after 35 fries (7 hours) and 45 fries (9 hours) respectively in oil with the tocopherol or rosemary treatment. This clearly shows that the rosemary antioxidant helps to extend the shelf life of frying oil. As has been shown in the majority of studies conducted on frying oils, TPM's increase over time when the number of frying operations increases (Urbančič et al., 2014). Song et al. (2017) monitored the level of TPM in oil heated at 180 °C and used for frying chicken using two methods, column chromatography and measurement of TPM by the tester (Testo 270). The results of both methods were similar throughout the kinetics, showing that the identifying of TPM by the tester (Testo 270) is a reliable method that can replace time-consuming chromatographic methods. A study by Freire et al. (2013) reported that 6 hours of frying with untreated oil or fat can avoid excess polar compounds in the frying process and safeguard the quality of fried food. Li et al. (2021) monitored TPM's by tester (testo 270) during several days of frying of chips in soybean oil treated with rosemary antioxidant, with synthetic antioxidant (TBHQ), and compared to the control without treatment. The Results showed that during 5 days of frying, the percentage of TPM of oil treated with rosemary

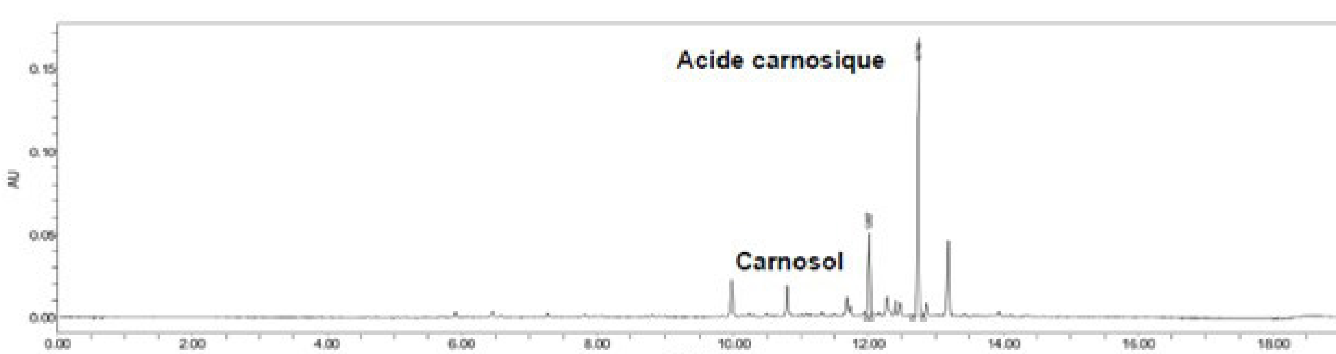


**Figure 1**. Chromatogram of rosemary antioxidant extract analyzed by UHPLC.

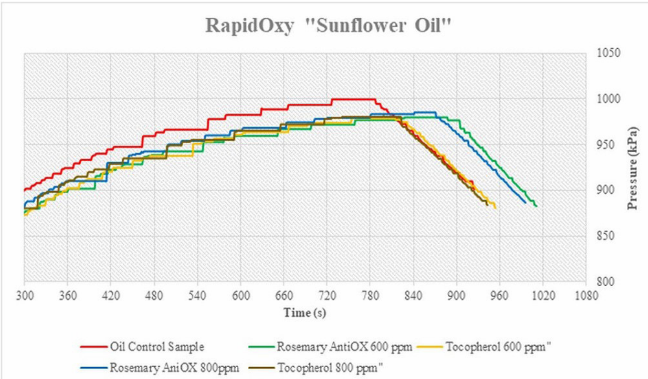


**Figure 2**. Monitoring of oxidation stability of sunflower oil by RapidOxy.

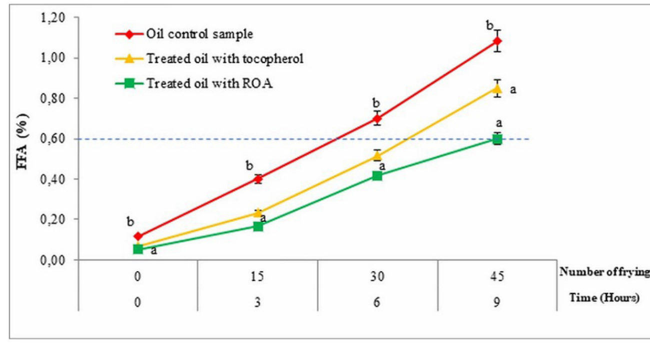


**Figure 4**. Oxidation kinetics of oil for the frying of chicken wings by measurement of FFA. Points with identical letters can be considered statistically identical.

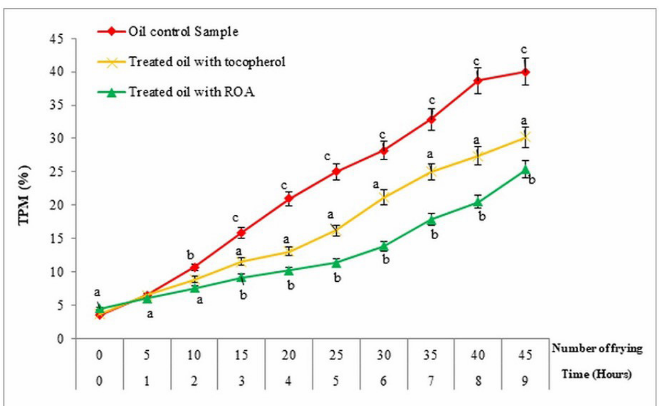


**Figure 3**. Oxidation kinetics of frying oil on chicken wings by measurement of TPM. Points with identical letters can be considered to be statistically identical.

antioxidant was lower than the oil treated with TBHQ which in turn was lower than the untreated oil (Li et al., 2021). This shows that the rosemary antioxidant was more effective than TBHQ in preventing the oxidation of frying oil.

### *3.4 Free Fatty Acids (FFA)*

FFA analysis was performed on samples taken every three hours during the frying process (Figure 4). After 30 fries (After 6 hours), the untreated oil already exceeds 0.6%. The oil treated with tocopherol reaches the same level around 35 frying (After 7 hours), while the oil treated with the rosemary antioxidant reaches 0.6% after 45 frying (After 9 hours). This once again proves the effectiveness of the antioxidant in fighting against compounds that degrade the quality of the frying oil. This has already been proven by a study that stated that rosemary extract decreases the FFA content of palm oil throughout a period of 5 days of frying potatoes at 180 °C (Guo et al., 2016). The same effect was observed by Urbančič et al. (2014) who used rosemary extract in sunflower oil which inhibited nearly 50% of FFA after 20 frying cycles compared to a control oil and also protected the oil from hydrolysis. Furthermore, another study monitored the evolution of the content of free fatty acids in untreated soybean oil used in the frying of different kinds of foods (Jorge & Janieri, 2008). The reported values were 0.11% to 0.36% after 15 hours of frying, which is consistent with what we found after 15 hours of frying chicken wings in untreated oil.

### *3.5 Peroxide Index (PI)*

The peroxide value is also one of the oxidation markers that evaluates the effectiveness of the tested rosemary antioxidant (Figure 5). The results presented clearly show a significant difference between untreated and treated oil. During the first 30 fries, which lasted 6 hours, the peroxide index of sunflower oil treated with rosemary antioxidant increased slowly, it barely reached 9 meq$O_2$/Kg, while that of the untreated oil reached 23 meq$O_2$/Kg. The PI value of the oil treated with tocopherol always remains average reaching 17 meqO2/Kg. This difference in PI remains significant even after 45 fries (9 hours). Therefore, the presence of the rosemary antioxidant in the oil delayed the oxidation reactions and inhibited the formation of peroxides. Chen et al. (2013) also evaluated the degree of sunflower oil oxidation by identifying the peroxide index in the absence of and in the presence of rosemary extract compared to synthetic antioxidants; BHA and BHT, at 60 °C for a period of 21 days. The results showed that the PI increased with the storage time, and in particular concerning the control. The use of all those antioxidants slowed down the rate of peroxide formation, but the effect of the rosemary extract was superior to that of BHA and BHT (Chen et al., 2013). These results concord with those of Babovic et al. (2010) who reported that on the basis of the PI, rosemary extract was more effective against sunflower oil oxidation than BHA after 12 h of storage at 98 °C. In addition, another study confirmed that rosemary extract can protect palm oil from oxidation and effectively reduce the peroxide value even at very high temperatures (180 °C), unlike synthetic antioxidants during potato frying Guo et al. (2016).

Rosemary extracts with their phenolic diterpenes (carnosic acid and carnosol), which have a great antioxidant activity, have not only an effect on lipid oxidation but also a great positive impact on human health. Several scientific studies have revealed diverse activities of rosemary and other plants including antioxidant,

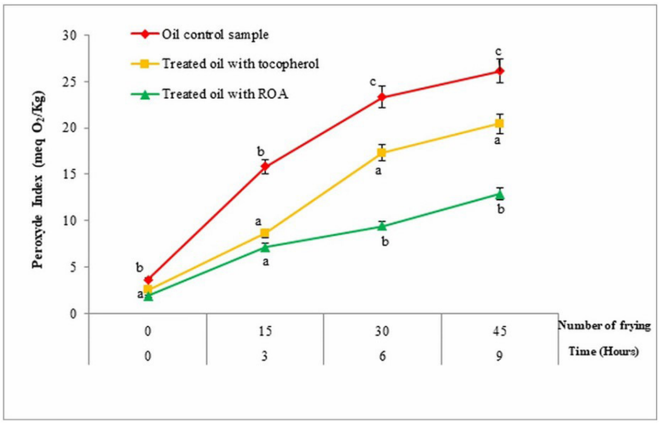


**Figure 5**. Peroxide index of sunflower frying oil with fried chicken wings at 175 °C. Points with identical letters can be considered statistically identical.

antitumor, antiplasmodial, and antidiabetic effects, which has attracted the attention of the medical and pharmaceutical sectors (Alonso, 2019; Shafay et al., 2022). Anwar & Qadir (2021) also reported anticancer, anti-inflammatory, neuroprotective, and hepatoprotective activities of carnosic acid and carnosol (Anwar & Qadir, 2021). Moreover, rosemary had a therapeutic potential against Alzheimer's disease (Veenstra & Johnson, 2021), in addition to a large number of very positive effects on human health studied in depth by Jiang (2019). These beneficial effects can be transmitted to humans through the consumption of foods treated with these rosemary antioxidants, hence the interest in including them in our nutritional routine.

## 4 Conclusion

The stability of frying oils and consequently of fried foods helps preserve nutritional and olfactory characteristics by protecting the fatty acids and by reducing the level of oxidation. The TPM tester « Total polar materials », along with the analysis of free fatty acids and the peroxide index demonstrated the superiority of quality of the oil treated with the rosemary antioxidant compared to the untreated control. This rosemary antioxidant helps reduce lipid auto-oxidation and photo-oxidation and ensures good oil quality with a longer shelf life than the untreated control. It also has the capacity to improve the sensory characteristics of fried foods, reducing the sensation of rancidity and protecting their colors. Rosemary extract has anti-cancer activity along with many other positive effects on human health. Thus, it can represent an interesting alternative to synthetic antioxidants that present a certain toxicological risk, such as buthylated hydroxyanisole BHA, buthylated hydroxytoluene BHT and tert-butylhydroquinone TBHQ.

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