

SELECTION AND ROLE OF A MIX ROSEMARY (*Rosmarinus officinalis*) - PALMITATE ASCORBYL AS ANTIOXIDANT IN A FRYING PROCESS

SELECCIÓN Y FUNCIÓN DE UNA MEZCLA DE ASCORBIL PALMITATO-ROMERO (*Rosmarinus officinalis*) COMO ANTIOXIDANTE EN UN PROCESO DE FRITURA

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ABSTRACT

Background: Several investigations report to rosemary as a plant rich in bioactive components with antioxidant potential, in this work, a rosemary extract was obtained that combined with ascorbyl palmitate provides a synergistic protection to a high fat diet (palm olein). **Objectives:** The objective of this study was to evaluate the effect of the addition of two extracts of rosemary (*Rosmarinus officinalis*): AP10R and AP30R at three concentrations of 1000, 1500 and 2000 ppm, on the oxidative stability of palm oil subjected to accelerated oxidation conditions and in a frying process. **Methods:** Lipid peroxidation of palm olein with and without antioxidants was monitored by measuring the concentration of hydroperoxides and total polar compounds; the thermal stability of the phenolic compounds in the oil was evaluated by fluorescence spectroscopy. **Results:** The AP10R extract at 2000 ppm inhibited olein oxidation by 30% and 60% in terms of total hydroperoxide and polar concentrations, respectively. The AP30 extract at 2000 ppm had similar inhibition behaviors with values of 27% of total hydroperoxides and 54% by total polar compounds in a time from 20 to 25 h. **Conclusions:** The results indicated that heating reduces the concentration of polyphenols; this decrease was more evident in olein without antioxidants, reflecting the effect of the polyphenols of rosemary extract on the thermal stability of palm olein.

Keywords: Oxidative stability, rosemary (*Rosmarinus officinalis*), hydroperoxides, total polar compounds (TPC), fluorescence spectroscopy

RESUMEN

Antecedentes: Diversas investigaciones reportan al romero como una planta rica en componentes bioactivos con potencial antioxidante, en este trabajo, se obtuvo un extracto de romero que combinado con ascorbil palmitato brinda una protección sinérgica a un sistema alimenticio con alto contenido graso (oleína de palma). **Objetivos:** El objetivo de este estudio fue evaluar el efecto de la adición de dos extractos de romero (*Rosmarinus officinalis*): AP10R y AP30R a tres concentraciones de 1000, 1500 y 2000 ppm, sobre la estabilidad oxidativa del aceite de palma sometido a condiciones de oxidación acelerada y en un proceso de fritura. **Métodos:** La peroxidación lipídica de la oleína de palma con y sin antioxidantes fue monitoreada midiendo la concentración de hidroperóxidos y los compuestos polares totales; la estabilidad térmica de los compuestos fenólicos en el aceite se evaluó por espectroscopia de fluorescencia.

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Resultados: El extracto AP10R a 2000 ppm inhibió la oxidación de oleína en 30% y 60% en términos de la concentración de hidroperóxidos y polares totales, respectivamente. El extracto AP30 a 2000 ppm tuvo comportamientos similares de inhibición con valores de 27% para hidroperóxidos y 54% para el contenido de fenoles totales en un tiempo de 20 a 25 h. **Conclusiones:** Los resultados indicaron que el calentamiento disminuye la concentración de polifenoles; esta disminución fue más evidente en la oleína sin antioxidantes, reflejando el efecto de los polifenoles del extracto de romero sobre la estabilidad térmica de la oleína de palma.

Palabras clave: estabilidad oxidativa, romero (*Rosmarinus officinalis*), hidroperóxidos, compuestos polares totales (CPT), espectroscopia de fluorescencia

INTRODUCTION

Deep frying is a process where are important, the nature of the oils and the food, type of fryer, temperature, exposure time, the replacement of oil and the use of antioxidants, among others. In the frying, the food in contact with the oil releases high amounts of water, that catalyze the hydrolysis of acylglycerols generating free unsaturated fatty acids (RH), which in the presence of low oxygen concentrations initiate lipid peroxidation process, producing primary process compounds such as conjugated dienes. During the oxidation process, hydroperoxides (ROOH) are generated as intermediate indicators of oxidative deterioration, which can be monitored and expressed as peroxide value (PV). Hydroperoxides are decomposed into other species more oxidized as aldehydes, ketones and organic acids, which by successive reactions form highly oxidized compounds. The concentration of all this species can be measured over time and are called Polar Compounds Total (CPT), which are indicators of the final oil deterioration (1- 4).

The addition of antioxidants is the most commonly used form to retard lipid oxidation processes and extend the shelf life of oils and fats (5). Synthetic antioxidants BHT, BHA, TBHQ and PG, have begun to be replaced by natural antioxidants, found mainly in plants and spices (6). Natural antioxidants are mainly polyphenolic compounds that help to stabilize oils and food, trapping free radicals and retarding the oxidative processes. The rosemary extracts have been used in stabilizing food matrices with high oils content, helping to prolong the shelf life by stabilizing radicals formed during autoxidation reactions (7, 8). This extracts are constituted by two major phenolic compounds: carnosic acid and rosmarinic acid, that has a high antioxidant activity (9).

To increase shelf life of oils in complex systems such as frying, antioxidant synergistic mixtures are used. For example, because ascorbyl palmitate lipophilicity is widely used with rosemary extracts. Some reports suggest that the addition of ascorbyl palmitate in oil, alone or in combination with other antioxidants helps promote stability to phenomena of photo- oxidation and lipid peroxidation during storage and during the frying process, prolonging the life of the fried product, and improving the quality of oil used during the process with the consequent increase in times of frying (10).

However, few reports where the concentration of phenolic compounds as protective of oxidative process in frying oil palm are evaluated. Monitoring the concentration of these compounds in oils provides a good quality parameter of the efficiency of the antioxidant system used. A suitable technique for monitoring the oxidative stability of fats and oils is fluorescence spectroscopy; this methodology is one of the most promising for the analysis of complex food matrices; due to its high sensitivity. There are some reports on use of this methodology for the analysis of vegetable oils, but few studies of stability of phenolic compounds in edible oils subjected to high temperatures (11).

The objective of this study was to evaluate the synergistic effect of the addition of ascorbyl palmitate to a rosemary extract (AP10R and AP30R) to inhibit lipid peroxidation in a palm oil to apply in a frying process. To determine the effect of temperature on the concentration of phenolic compounds in the oil palm supplemented with antioxidants by fluorescence spectrophotometry. Finally evaluate the effect of the addition of the best concentration of rosemary extract-ascorbyl palmitate in stabilizing palm oil in frying chicken nuggets, through monitoring of the primary and secondary oxidation products.

MATERIALS AND METHODS

Preparation rosemary (*Rosmarinus officinalis*) extract

Dried rosemary leaves, grown in the department of Antioquia (Colombia), were pulverized in industrial cutting mill (Retsch®), then they were extracted with mechanical agitation using as solvent a solution of ethanol: propylene glycol. The ethanol extract obtained was filtered through a Whatman type (GF/A, 110mm) paper and excess solvent was distilled under vacuum at 40°C on a rotary evaporator and vehiculized in glicerol. In order to increase the solubility and stability of rosemary (*Rosmarinus officinalis*) extract was added ascorbyl palmitate dissolved in polysorbate 20 to a final concentration of 10 ppm (AP10) and 30 ppm (AP30) respectively. These solutions were studied at 1000, 1500 and 2000 ppm in palm oil, to select the best to use in frying of nuggets.

Composition of the rosemary (*Rosmarinus officinalis*) extracts

Determination of total phenol

Determination of phenols was performed by the colorimetric method Folin-Ciocalteu, described by Singleton and Rossi (1965), with some modifications (10). To 50 µL of the working solutions were added to 125 µL of Folin reactive and 400 µL of sodium carbonate 7.1% (w/v), adjusted with distilled water to 1000 µL, spectrophotometric reading at 760 nm was performed and compared to the standard curve using standard gallic acid (phenol). The results were expressed as mg gallic acid equivalents /g of dried sample (DS) (12).

Determination of carnosic and rosmarinic acid by HPLC

The content of carnosic acid (CA) and rosmarinic (AR) of the rosemary extract was determined by HPLC analysis. The ethanol extract of rosemary diluted in ethanol was filtered through a 0.45 µm pore size, before injection to the chromatograph. Quantification of both acids was performed on a LiChrospher® 100RP-18 column (5 M) 250 × 4. The mobile phase was programed as a linear gradient from 95% A (water 840 mL, 150 mL of acetonitrile and 8.5 mL of acetic acid) and 5% B (methanol) to 100% B in 40 minutes with a flow

of 0.6 mL/min and an injection volume of 20 µL. The identification of both acids was performed by comparison of retention times with those of pure standards. A liquid chromatograph (Shimadzu LC-20AD), equipped with a SIL-20A auto injector/HT, a communication module and CBM- 20A (PDA) SPD-M20A calibrated at 284 nm was used (13).

Phenolic acids by High-Performance Liquid Chromatography (HPLC)

The ethanolic extract of rosemary was filtered (0.45 µm pore size filter) prior to injection. The chromatograph was equipped with a SIL-20A auto injector/HT, a CBM-20A communication module and a SPD -M20A photodiode array detector (PDA) calibrated at 320 nm. Quantification was performed on a Merck RP18 column with a 5 µm particle size, 250 mm length and 4.0 mm diameter. The mobile phase was comprised of methanol and acidified water with 0.1% formic acid. The flow rate of the mobile phase was 0.6 mL/min, and the other conditions were 50°C and gradient elution. The UV-visible spectrum from 200 to 600 nm was measured for all peaks; identification and quantification of the compounds were performed using calibration curves for each of the phenolic acids (14).

Antioxidant activity of rosemary extract

The antioxidant activity of rosemary extract was evaluated by different methods lipophilic and hydrophilic.

Evaluation of free radical –scavenging activity by DPPH assay

The antioxidant activities of Rossemary extract (dissolved in MeOH) were assessed on the basis of the radical scavenging effect of the stable DPPH free radical. This procedure is carried out using 10 µL of the test compound and 990 µL of the methanol solution of DPPH • (20 mg/L). As reference, the same amount of DPPH was used and 10 µL sample solvent (MeOH). After 30 minutes of reaction at 25 degrees Celsius and in the dark, the absorbance is read at a wavelength of 517 nm. For each studied sample, the percent inhibition of the radical is calculated and the results are expressed as TEAC (Trolox Equivalent Antioxidant Capacity) values by constructing a standard curve using various concentrations of antioxidant Trolox (15).

Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was determined using the following methodology: 3 mL was prepared from the following solution: 21 μ L of a 10 μ M solution of fluorescein, 2899 μ L of 75 mM phosphate buffer (pH 7.4), 50 μ L of 600 mM 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) and 30 μ L of extract. Fluorescence was recorded on a Perkin Elmer LS45 spectrofluorometer with a thermostated multicell. The ORAC value μ mol Trolox/g DS was calculated by a calibration curve using different concentrations of Trolox® (16).

Fatty acid composition of palm oil (*Elaeis guineensis*) by GC-MS

For the study, Ricapalma® oil was used. The fatty acid profile of the palm oil was performed on an Agilent gas chromatograph 6890N coupled to an Agilent selective detector MS 5973N and equipped with a split/splitless. The software used to calculate all parameters was MSD ChemStation D 02.00.275 Copyright® Agilent Technologies 1989-2005. For determining saturated and unsaturated fatty acids as methyl esters NIST database 2005 was used (16).

Oxidative stability of palm oil (*Elaeis guineensis*)

Stability study of palm oil: AOM method

To evaluate the best rosemary extract mixture supplemented with ascorbyl palmitate 10 and 30 ppm; accelerated AOM method, in which the appearance of hydroperoxides was monitored until 100 peroxides value (PV) (1). To 20 mL palm oil is added AP10R and AP30R rosemary extract until final concentrations of 1000 ppm (AP10R1000 and AP30R1000), 1500 ppm (AP10R1500 and AP30R1500) and 2000 ppm (AP10R2000 and AP30R2000). As a control, a sample of palm oil without antioxidant was taken. The samples were subjected to a temperature of $99 \pm 0.1^\circ\text{C}$ and an airflow of 1150 mL/min.

Determination of peroxide value (PV)

The peroxide value was determined by the method described by Shantha *et al.*, with some modifications (15). This method is based on the ability of lipid peroxide to oxidize Fe^{2+} to Fe^{3+} . To 0.03 g of sample was added 3.5 mL of chloroform: methanol (7:3), the mixture was stirred for 10 seconds. Then 1 mL was taken from the mixture and added 50 mL of

Fe^{3+} solution, prepared from FeSO_4 (0.144M) and BaCl_2 in HCl (0.4M), and 50 mL of a solution of NH_4SCN (0.44M), this mixture was incubated for a period of 20 minutes in the dark; past which time the absorbance was determined at a wavelength of 510 nm in a spectrophotometer Jenway® 6405 UV/Vis. The results were expressed as milliequivalents of oxygen/kg oil (oxygen meq/kg oil).

To evaluate the effect of rosemary extract supplemented with ascorbyl palmitate previously selected to the frying process, the appearance of total polar compounds (TPC) and peroxides value (PV) were studied (17).

Determination of total polar compounds percentage (% TPC)

Content total polar compounds was determined according the IUPAC method (International Union of Pure and Applied Chemistry) under the standard 2507, 1987, using the adsorption chromatography in silica gel column as solid phase and a solvent mixture (hexane: ethyl ether (90:10 v/v) as mobile phase to elute the non-polar compounds (unaltered triglycerides), and so then obtained by weight difference content of polar compounds and their respective percentages (1).

The antioxidant effect on the process of palm oil oxidation was evaluated by the decrease of total phenols in the time. The antioxidant rosemary-ascorbyl selected was added to the oil to 180°C for a period of 4 hours and at the end was measured the content of total phenols by fluorescence spectroscopy.

Frying tests

120 g chicken nuggets Kokoriko® were subjected to a batch process of deep frying in 750 mL of palm oil (with and without addition of antioxidant), preheated to 180°C using a home fryer. The nuggets were submerged in oil for a period of five minutes, five each batch frying oil sample was taken to perform the analysis of the content of hydroperoxides (PV) and percentage of total polar compounds (% TPC) in the oil. The objective of the work was to enhance the activity of rosemary extract with small concentrations of ascorbyl palmitate in an addition value that synergistically enhances the protection of the frying oil. For this, white without antioxidant is used. The selection and dosage of antioxidant that was used in the assay was performed according

to the results of the accelerated stability study described in the previous section.

Fluorescence spectroscopy

Fluorescence spectra of the diluted oil (100 μ L in 6 mL of propanol) were obtained on a Perkin Elmer LS45 spectrofluorimeter equipped with a xenon lamp and two graduated monochromators, one for broadcast and one for excitation. An excitation spectrum was selected to 330 nm emission for fluorescence measurement of phenolic compounds present in the oil palm oil with and without addition of rosemary extract (10, 13, 18). The slit was set as 10 nm to filter 1% and the fluorescence was measured in intensity units.

Kinetic study of oxidative degradation of the phenolic compounds

A change in the quality of phenolic compounds can be measured by the appearance or disappearance of one or more quantifiable indices, symbolized by A (total phenols); the rate of appearance or disappearance of A can be represented by the Equation (1).

$$rA = \frac{dA}{dt} = K[A]^m \quad (\text{Equation 1})$$

where K is the rate constant and m is the apparent order of reaction. Then when m is 0, 1 and 2 the rate equation become in Equations (2)-(4) respectively.

$$A = A_0 - Kt \quad m = 0 \quad (\text{Equation 2})$$

$$A = A_0 e^{-Kt} \quad m = 1 \quad (\text{Equation 3})$$

$$\frac{1}{A} = \frac{1}{A_0} - Kt \quad m = 2 \quad (\text{Equation 4})$$

Statistical analysis

To identify significant differences between treatment groups at different doses (1000, 1500 and 2000 ppm) analysis of variance (ANOVA) was performed with a confidence level of 95%. According to the results obtained for the formation of hydroperoxides and total polar compounds, the equations that model the behavior through regressions are

established. Statgraphics Centurion XV statistical software version 15.2.06 was used.

RESULTS

Composition and antioxidant activity of rosemary extract

The results of the composition of rosemary extract are showed in Table 1.

Table 1. Composition of rosemary extract.

ANTIOXIDANT CAPACITY VARIABLE	VALUE
Total phenol content ¹	27.6
Rosmarinic acid ²	27533.9
Carnosic acid ³	245.7
ORAC ⁴	4375.2
DPPH ⁵	623.0

¹Gallic acid equivalent/g extract. ²mg Rosmarinic Acid/L. ³mg Carnosic acid/L. ⁴ μ mol Trolox/g. ⁵ μ mol Trolox/g.

The content of carnosic acid in rosemary extract obtained is much lower than previously reported in some rosemary extracts from other latitudes.

Fatty acid profile palm oil and selection of the concentration of rosemary

Palm oil were detected 10 major fatty acids, with a ratio of % unsaturated / saturated (I / S = 1.0) and with long chains of 10 to 20 carbons, were detected. Table 2 shows the percentage of fatty acids of palm oil, where the oleic acid (ω 9) (48.6%), palmitic (35.1%), stearic (6.2%) and palmitoleic (1.8%) are the major components and very stable chemically.

Table 2. Fatty acid profile of palm oil.

FATTY ACID	PERCENTAGE (%)*
Pentadecylic C10:0	0.53 +/-0.02
Lauric C12:0	0.93 +/-0.04
Palmitic C16:0	35.11 +/-1.78
Heptadecanoic C17:0	0.21 +/-0.01
Stearic C18:0	6.23 +/-0.31
Arachidonic C20:0	0.41 +/-0.02
Behemic C22:0	0.12 +/-0.006
Palmitoleic C16:1	1.80 +/-0.09
Oleic C18:1	48.63 +/-2.47
Linoleic C18:2	0.06 +/-0.003

* Data are the mean +/-standard mean error, n=3

Figures 1A and 1B show the behavior in the formation of hydroperoxides (PV) in palm oil

supplemented with rosemary extract AP10R to 1000 ppm, 1500 ppm, 2000 ppm and AP30R, to 1000 ppm, 1500 ppm and 2000 ppm, respectively.

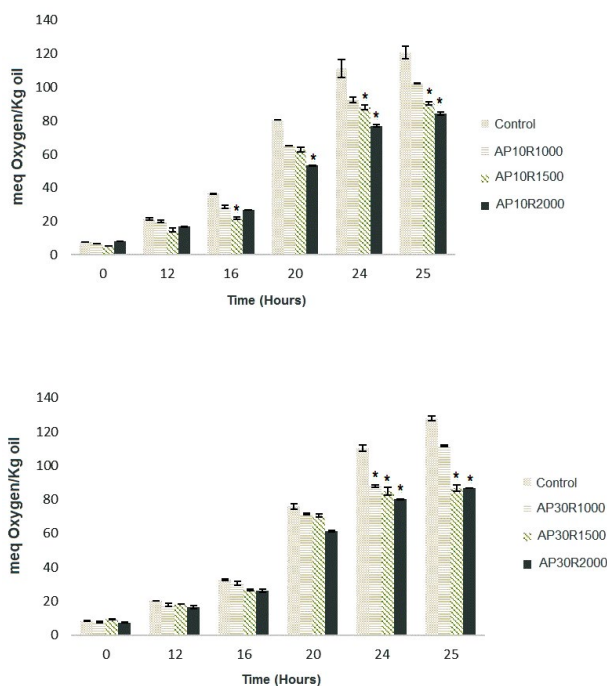


Figure 1. A. Evolution of hydroperoxides formation in palm oil with added AP10R 1000, 1500 and 2000 ppm and **B.** Evolution of hydroperoxides formation in palm oil with added AP30R 1000, 1500 and 2000 ppm. Data are mean \pm standard mean error of 3 separate experiments. The asterisk indicates a significant difference between controls and treated, $*p < 0.05$.

To determine the optimal concentration of the antioxidant by the AOM method, are determined the times (hours) to reach a peroxide value equal to 100 meq O₂/kg oil, for each concentration of antioxidant and calculating a ratio between the control and each concentrations and the higher ratio giving the best antioxidant concentration value for use in the frying process (19).

As shown in Figures 1a and 1b, the content of hydroperoxides in palm oil supplemented with rosemary extracts codified as AP30R and AP10R respectively, increases exponentially throughout the test. Additionally, for both antioxidants for AP30R and AP10R one dose response, ie, the higher the concentration of the antioxidant content smaller effect is observed hydroperoxides, that is, the higher the concentration of the antioxidant, lower content of hydroperoxides. This effect is more evident in the final hours of experimentation where the

hydroperoxides forming speed is lower than the other treatments after the addition of antioxidants AP30R2000 and AP10R2000.

The addition of AP30R and AP10R at a dose of 2000 ppm reduces the production of hydroperoxides; within 25 hours of experimentation, it is reduced by 32.32% and 30.21%, respectively. AP30R and AP10R antioxidants at a concentration of 1000 ppm reduce the content of hydroperoxides in 15.45% and 12.76%. Similarly, treatments 1500 ppm reduced 25.26% and 32.16%, respectively.

Table 3 show equations that modeling the production of hydroperoxides in function of time and the calculated P value for each of the treatments used. These equations were selected according to the correlation coefficient (r^2). The results indicated that the treatment with highest protection palm oil is AP10R2000. The calculated parameter is a good criterion for selecting the best concentration of antioxidant for the development of frying tests.

Antioxidant effect in the oxidative process

However, it is necessary to know the quantitative effect exerted by the polyphenol antioxidants added as AP10R2000 on palm oil. Previous reports in olive oils study the evolution of polyphenols tyrosol and hydroxytyrosol oils subjected to high temperatures fluorescent methods (20, 11). In the present study, monitoring the phenol concentration in the palm oil were proposed, with and without addition of AP10R to 2000 ppm, during heating at 180°C for a period of 4 hours, by fluorescence spectroscopy.

Table 3. Model equations hydroperoxide formation in palm oil, and protection value (P) for AP10R and treatments AP30R, 1000, 1500 and 2000 ppm.

Treatment	Equation	r^2 *	$P(t_m/t_c)$
Control AP10R	$VP = \exp(1.9076 + 0.114935^*t)$	0.989	1.000
AP10R1000	$VP = \exp(1.79083 + 0.111849^*t)$	0.980	1.098
AP10R1500	$VP = \exp(1.50039 + 0.119428^*t)$	0.956**	1.134
AP10R2000	$VP = \exp(1.91124 + 0.0978061^*t)$	0.962**	1.202
Control AP30R	$VP = \exp(1.91674 + 0.113372^*t)$	0.965	1.000
AP30R1000	$VP = \exp(1.85893 + 0.109794^*t)$	0.960	1.055
AP30R1500	$VP = \exp(2.0397 + 0.0964459^*t)$	0.931**	1.122
AP30R2000	$VP = \exp(1.83218 + 0.103557^*t)$	0.957	1.129

* r^2 : Linear correlation coefficient; ** The asterisk indicates a significant difference compared with controls ** $p < 0.05$.

Previous studies have found that the fluorescence intensity measured by an excitation spectrum to 330 nm emission with a peak intensity in a range

of 280-330 nm, corresponding to the presence of phenolic compounds in vegetable oils such as olive, wherein fluorescence intensities is proportional to the concentration (21, 11). The exact position of the maximum intensity in the excitation spectrum may vary between different oils studied, due to differences in the composition (11).

In Figures 2A and 2B are presented the excitation spectra to 330 nm palm oil emission with and without addition of AP10R to 2000 ppm, subjected to heating, respectively.

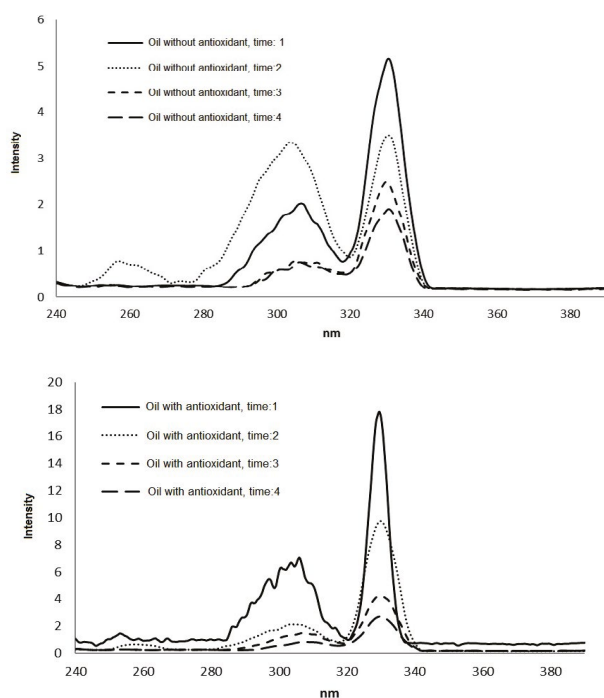


Figure 2.A. Excitation spectrum to 330 nm palm oil emission without rosemary extract added and **B.** Excitation spectrum to 330 nm palm oil emission with addition of AP10R to 2000 ppm.

As can be seen, the excitation spectrum has a maximum intensity to 330 nm in palm oil with and without addition of extract AP10R; this peak is indicative of the polyphenol content in the samples analyzed.

Table 4 shows the areas calculated for those covered peaks between 320 and 339 nm excitation spectra to 330 nm at every hour of rehearsal for palm oil with and without addition of rosemary extract subjected to heating.

In both samples was observed a decrease in the area under the curve to an increased heating time, which is related to the oxidation of phenolic

compounds present as result of their action in the inhibition of the formation of hydroperoxides in the oil.

Table 4. Peak areas calculated of the excitation spectrum to 330 nm of emission between 320 and 339 nm, for palm oil with and without adding antioxidant during warming.

Treatment	Time (Hours)	Peak area
Oil without antioxidant	1	58.60
Oil without antioxidant	2	40.52
Oil without antioxidant	3	28.99
Oil without antioxidant	4	22.64
Oil with antioxidant	1	130.11
Oil with antioxidant	2	109.79
Oil with antioxidant	3	50.23
Oil with antioxidant	4	31.77

From the above results, AP10R extract at a concentration of 2000 ppm to proceed with the frying tests was selected.

Frying tests

In this study was selected 26% of total polar compounds (TPC) like maximum limit oil rejection.

In Figure 3 is presented the behavior of the formation of total polar compounds % TPC in palm oil with and without addition of AP10R.

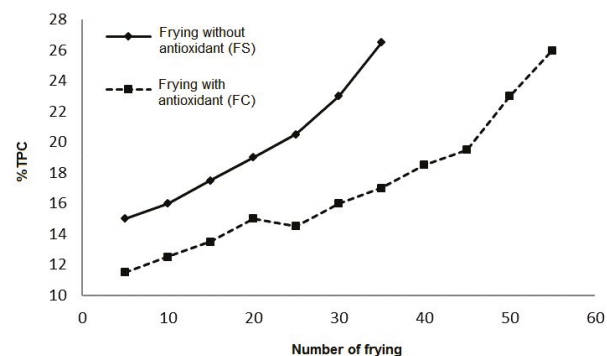


Figure 3. Evolution of total polar compounds formation (% TPC) in palm oil, frying subject to conditions, with and without addition of AP10R to 2000 ppm. Data are mean +/- standard mean, n=3.

As can be seen from the graph of the rate of increase of % TPC in oil with the addition of AP10R is lower than the control oil. In this respect, the control sample reached the rejection concentration in frying 35, while the sample with addition of AP10R reached in the frying 55.

The behavior identified in the concentration of total polar compounds, interacts well with the observed in the formation of hydroperoxides in the oil with and without addition of AP10R (See Figure 4).

In the oil without added antioxidant the rate of formation of hydroperoxides is greater than in the oil with AP10R to 2000 ppm. The above demonstrates the effect of rosemary in oxidative stabilization of palm oil for frying trials; various studies have shown the efficiency of such extracts in stabilizing radicals formed during the propagation stage of lipid peroxidation (9).

Thus the concentration of hydroperoxides in the oil with AP10R remains almost constant over a period of 45 chips which increases exponentially.

This behavior has been previously reported in the literature, where, in the initial stages of oxidation, the concentration of hydroperoxide remains constant, due to the action of antioxidants; once the antioxidant is exhausted, the growth rate of this product increases exponentially (19). In the case of oil without antioxidant added the growth period starts from frying number 15, which is reflected in the shortest period of oil life under the conditions studied.

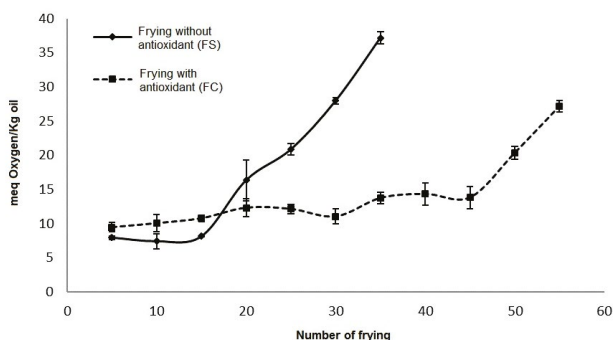


Figure 4. Evolution of hydroperoxide formation in palm oil, under frying conditions with and without addition of AP10R to 2000 ppm. Data are mean \pm standard mean, $n=3$.

Analogously as described in the stability study of palm oil it proceeded with calculating the value of protection.

Table 5 shows the equations that modelling the behavior of hydroperoxide formation in palm oil under frying conditions, with and without the addition of antioxidant and the protection value defined as the number of chips (f_m) it takes the sample AP10R at 2000 ppm to reach a peroxide value of 100 meq peroxide oxygen/kg of oil, compared to the number of chips (f_c) that takes the control displays the same value.

Table 5. Model equations hydroperoxides formation (PV) in palm oil, under frying conditions, and protection value (P) for treatment AP10R to 2000 ppm.

Treatment	Equation	r^{2*}	$P(f_m/f_c)$
AP10R Control	$PV = \exp(2.58986 + 0.0185094^*t)$	0.9851	1.0000
AP10R2000	$PV = \exp(2.39974 + 0.012593^*t)$	0.9752	1.6084

* r^2 : Linear correlation coefficient. Values do not present a significant difference compared with controls ** $p < 0.05$.

The estimated value of protection is possible to observe that the addition of the antioxidant extends the life of palm oil in a 61%. The above results demonstrate the efficiency of this antioxidant in the stabilization of the oil, so that positions as good candidate for stabilizing lipid matrices rather than synthetic antioxidants customarily employed for this purpose.

Kinetic of degradation of phenolic compounds

Taking as reference the exciting spectrum of Figures 2A and 2B, the values of phenolic compounds present in the table 6 are obtained.

Both treatments decreased the concentration of phenolic compounds, oil with added antioxidant AP10R to 2000 ppm presented highest concentration thereof along the four hours of heating.

Table 6. Values of phenolics compounds in the palm olein with and without addition of AP10R to 2000 ppm.

Time of heating (h)	Phenolics compounds oil with antioxidant (AP10R 2000 ppm)	r^{2*}	Phenolics compounds oil without antioxidant (AP10R 2000 ppm)	r^{2*}
1	22.15	0.989	16.54	0.931
2	10.75	0.974	11.27	0.945
3	7.51	0.962	4.51	0.913
4	8.02	0.973	2.4	0.948

*Linear correlation coefficient (r^2), $n=2$.

DISCUSSION

Natural antioxidants are mainly polyphenolic compounds that help to stabilize oils and food, trapping free radicals and retarding the oxidative processes. The rosemary extracts have been used in stabilizing food matrices with high oils content, helping to prolong the shelf life by stabilizing radicals formed during autoxidation reactions.

Different methodologies have been proposed to evaluate antioxidant capacity; ORAC assay is a HAT (Hydrogen Atom Transfer) method that measures the capability of antioxidants to scavenge with peroxy radicals by hydrogen donation. While that TEAC is based on single electron transfer (SET) mechanisms in which the antioxidant compound tested donates an electron to the DPPH[•]. In both techniques extracts of rosemary have greater antioxidant activity than extracts of others Lamiaceae as *Melissa officinalis* (476.60 $\mu\text{mol Trolox/g}$ of extract) and (253.80 $\mu\text{mol Trolox/g DS}$) (22). Polyphenols are secondary metabolites responsible of the antioxidant activity of plant extracts, by their ability to react with reactive oxygen species by these mechanisms; specifically phenolic acids as caffeic, chlorogenic, *p*-coumaric and rosmarinic reported in these rosemary extracts. The results of total phenol content and rosmarinic acid in the extract of study are minors that obtained in different ethanolic extracts of rosemary in Mexico and extracted in other conditions, where reported total phenol content of 109.50 mg GAE/g extract, 37525 mg RA/g (18). Whole chlorogenic acid (8.6 mg/g and *p*-coumaric acid 9.4 mg/g) reported to this cultivar are very high (22).

The differences in the concentration of bioactives of rosemary may be due to different factors such as the region of origin and environmental conditions (altitude and latitude) and fundamentally the solvents and extraction methods used.

Oxidative phenomena of oils are the main cause of loss of sensory and nutritional quality of foods (21). These consist of a series of reactions self-catalytic, which are divided into three steps: initiation, propagation and termination. During the initiation phase, lipid radicals (R^{\bullet}) are generated by homolytic abstraction of a hydrogen adjacent a double bond of an unsaturated fatty acid; during propagation lipid radical (R^{\bullet}) reacts with triplet oxygen ($^3\text{O}_2$) to form peroxy radicals (ROO^{\bullet}) which react with new lipid molecules (RH) to form hydroperoxides (ROOH)

(23). In the initial stages of oxidation reaction rate is low, even more in the presence of antioxidants; once they are consumed, the rate of production of hydroperoxides rises, during the propagation stage, reaching a point at which its decomposition begins at the end oxidation products (21).

Palm oil composition reflect a high stability with a relation of unsaturated/saturated ($U/S = 1.0$) and likewise, reflect the stability against oxidative processes such as frying and longer life compared with other oils (24). However, rosemary extract enriched with polyphenols antioxidants may react in the oxidative process with reactive oxygen species and extending the useful life of fats and oils, an effect that can be monitored by accelerated methods as Active Oxygen Method (AOM) and Rancimat. AOM test involves peroxide values analyses that are compounds of the early stage of lipid oxidation. In order to compare the effect of the antioxidant doses used in the stabilization of palm oil, the P (tm/tc) value is calculated, where (tm) is the time that is taken by a sample with antioxidant to reach a concentration of 100 meq oxygen/kg of oil, with respect to the control time without antioxidant (tc), A P value greater than 1 is indicative of an antioxidant protective effect. Rosemary extract at concentration of 2000 ppm enriched with ascorbyl palmitate at a concentration of 10 ppm increases shelf life in a 32%. In this study a dose response was not found with respect to the amount of ascorbyl palmitate, a very particular phenomenon of this chemical species.

The effect of the antioxidant during oxidative processes has been little studied. Fluorescence spectroscopy can provide a good approximation for determining the oxidative status and quality of fats and oils. Previous studies have found that the fluorescence intensity measured by an excitation spectrum to 330 nm emission with a peak intensity in a range of 280–330 nm, corresponding to the presence of phenolic compounds in vegetable oils such as olive, wherein fluorescence intensities is proportional to the concentrations (21, 11). The exact position of the maximum intensity in the excitation spectrum may vary between different oils studied, due to differences in the composition (11).

The application of this method has been very well received, because the phenolic compounds are fluorescent molecules, and the oxidation of these compounds is followed through this methodology. Comparing between samples each time studied,

is possible observe that the areas calculated in the sample with added AP10R, are higher than those found in the control sample (oil without antioxidant), which is indicative of the contribution of phenolic compounds in the extract of rosemary added. This result correlates well with the results of stability testing of palm oil, in which could be found that adding rosemary extract increased oxidative stability and lifetime of palm oil.

On the other hand, the oxidation of frying oil depends on many factors such as the nature of the oil, the frying temperature and type of food, among others. Therefore, defining the end point of the use of oils in the frying process is complex. However in many countries a value between 25 and 27 of total polar compounds (TPC) is used as the end point of the process.

The formation of total polar compounds (TPC), in edible oils has been linked to primary and secondary fatty acid oxidation which occurs during the frying process (24); the concentration of these compounds is considered a quality index of oils, because the oxidized products formed during oxidation of the oil, are associated with adverse effects on human health (25). The estimated value of protection by the addition of the antioxidant extends the life of palm oil in a 60.84%. These results demonstrate the efficiency of this antioxidant in the stabilization of the oil and are a good candidate for stabilizing lipid matrices rather than synthetic antioxidants. Future studies could be carried out under the same methodology, but this time using other oils that are used both in industry and for mass consumption.

CONCLUSIONS

This study demonstrates the protective effect of rosemary extract–ascorbil palmitate against lipid peroxidation phenomena. Concentrations of rosemary extract studied demonstrated a dose–response effect in increasing the stability of palm oil.

The results obtained by fluorescence spectroscopy allowed to determine that warming decreases the concentration of polyphenols in the oil palm, with and without addition of AP10R rosemary extract. This decrease is related to the protective action of the polyphenols from the antioxidant, against thermo-oxidation phenomena experienced by the oil under the conditions studied, and evidenced through decay in the fluorescence intensity of the excitation spectrum emission to 330 nm.

The treatment AP10R to 2000 ppm proved to be the better of the two prototypes studied, which was selected to proceed with the trials frying, where it extended the life of the oil palm in 30.38% in terms of training percentage of total polar compounds (% TPC), and a 60.84% in terms of hydroperoxides formation (PV), as protection value, compared with the oil without added antioxidant (control).

Finally, the mixture of antioxidants is a good alternative for use in complex systems such as fried processes; because of the possible synergistic effect between its components, which must be supported experimentally before application.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHORS' CONTRIBUTIONS

The authors participated: drafting the article, revising it critically for important intellectual content, making substantial contributions to conception and design, acquisition of data, analysis and interpretation of data and giving final approval of the version to be submitted and any revised version.

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