# Kinetic modeling and evaluation of free radical-scavenging behavior in oils: application to four tropical and subtropical fruits in a DPPH system

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## Abstract

A kinetic model was developed to represent the radical-scavenging capacity (RSC) for the oxidation of oils. The model was developed using second-order rate constants ($k_2$) to represent the RSC. The RSC of the oils can be compared by the $k_2$ values indicating their scavenging capacity. Furthermore, the $k_2$ values can be used to determine the decay kinetic of oils prone to attack by free radicals and to determine the amount of antiradicals that should be added as protective agent to these oils. As a case study, the antiradical capacity of four oils produced from tropical and subtropical fruits (avocado, nance, palm and peach palm) were studied using the DPPH• assay.

**Keywords:** antioxidant; oil; fruit; DPPH.

**Practical Application:** Recently, the search for fruits with good sources of natural antioxidants, for example with high lipid contents, has stimulated studies that evaluate the antioxidant capacity of the extracted oils. Still, the scientific information on the functional potential of palm oils native to the Amazon is few, especially those that are used as sources of food in this region. Vegetable oils, especially native species from Brazil, may fill this gap, in addition to generating new alternatives of use, which will add value to these fruits, encouraging their consumption.

## 1 Introduction

The antioxidant capacity of phenolic compounds has become of interest because of its chemoprotective effect against degenerative diseases, such as cardiovascular and some neurological diseases, as well as its ability to inhibit lipid oxidation in food (Bubonja-Sonje et al., 2011). The antioxidants in oils have been widely studied by several authors, because they are important in the stabilization of free fatty acids and in the antioxidant activity of phenols and other compounds present in the oils. Above all, food quality, as well as nutritional value, are affected by changes in lipid structures (Ramalho & Jorge, 2006; Ribeiro & Jorge, 2017).

The antioxidant action is one of the biological activities originating from essential oils, being responsible for neutralizing the oxidative damages in the cells. This damage results in the proliferation of a chain reaction that damages the biological system, causing a series of pathologies (Viana et al., 2014; Miranda et al., 2016).

No research has been found that reports the radical elimination capacity of several oils using a kinetic model. The elimination by radical-scavengers (R •) can be summarized as (Equations 1, 2 and 3):

$$R• + OE \rightarrow R-H + A• \tag{1}$$

$$R• + A• \rightarrow R-A \tag{2}$$

$$A• + A• \rightarrow A-A \tag{3}$$

where: OE is a scavenger of the oil; A• is radical; R• is a radical such as DPPH•.

The equations confer with the study by Rufino et al. (2009) on the behavior of free radicals in the DPPH system in some fruits of the Brazilian Northeast. The newly formed radical (A•) accompanies radical-radical interaction to generate stable molecules via radical disproportion (shock of radicals abstracted from one atom by one radical to another - Equations 2 and 3), even if these secondary reactions are very impaired (Aruoma, 1998; Rufino et al., 2009). It is essential to emphasize that when studying oils, there are numerous possible species of elimination of radicals in the extracts.

Beverages and foods that have high antioxidant capacity are low in fat. However, there are still certain foods of plant origin, particularly walnuts, which are not only high in fat but also have considerable antioxidant capacity. There are studies that indicate that the consumption of macadamia, walnut and chestnut are favorable in reducing the risk of cardiovascular diseases, since they have an excellent relation between polyunsaturated fatty acids omega 3 and omega 6 (Oliveira et al., 2009; Freitas & Naves, 2010).

A large majority of studies have dealt directly with extracts of the non-defatted raw material, although some of them have analyzed the antioxidant capacity of oils or lipophilic compounds.

However, the contribution of the two main fractions of oil and defatted matter to their total antioxidant capacity is unknown. In addition, there are studies that reveal the possibility of the oil interfering with the determination of the antioxidant potential of samples rich in fat (Arranz et al., 2008).

This work has the objective of developing a kinetic model to represent the RSC for the oxidation of oils. As a case study, this work also aims to characterize the elimination capacity of free radicals of avocado oil, nance oil, palm oil, peach palm oil and olive oil using DPPH • to evaluate its potential as a source of antioxidants, comparing the kinetic approach.

## 2 Materials and methods

### 2.1 Samples

Nance (*Byrsonima crassifolia*) was collected at Embrapa Experimental Station at Paraipaba, Fortaleza and Pacajus, CE, Brazil. Peach palm (*Bactris gasipaes*) was collected at Rio Branco, AC, Brazil. Avocado (*Persea americana*) and palm (*Elaeis guineensis*) were collected at Fortaleza, CE, Brazil. Olive oil (*Olea europaea*) was obtained from a local supermarket and was used as reference oil. 2,2-Diphenyl-1-picrylhydrazyl (DPPH˙) was supplied by Sigma (St. Louis, MO). All other reagents were of analytical grade and were supplied by Vetec Quimica Fina (Duque de Caxias, RJ, Brazil).

### 2.2 Oil extraction and extracts preparation

Before the oil extraction, the fruits were lyophilized, showing the antioxidant capacity of the defatted sample and the oil (Rufino et al., 2011a). 0.5 g of lyophilized test tube material was added, and 20 mL of petroleum ether was added. The tube was stirred at room temperature for 20 min and then centrifuged at 2500 g for 10 min. The supernatant (oil phase + solvent) was recovered.

### 2.3 Free radical scavenging by DPPH• assay

In this research we chose to use the DPPH method for its efficiency in foods of lipophilic nature.

DPPH was dissolved in ethyl acetate (1: 1 v / v) as described by Espín et al. (2000). The experiments were carried out on freshly prepared solutions of radical, which, without radical scavengers were stable for more than 1 day. The use of ethyl acetate was necessary to allow the increase of the solubility of the extract, reaching the pseudo-first order test conditions, at which the initial concentration of the oil extraction was much lower than the initial radical concentration (Equation 4):

$$\left( [DPPH\bullet]_0 >> [OE]_0 \right) \tag{4}$$

The activity of removal of free radicals from oils was measured using the method described by Brand-Williams et al. (1995), with adaptations. A 0.06 mM solution of DPPH • in ethyl acetate was prepared and an aliquot of ± 0.1 mL of the antioxidant solution/oil extract was added to ± 3.9 mL of the DPPH • solution. The conditions of the anti-radical activity test were as follows: avocado 0.05-0.2 mL; nance 0.1-0.3 mL; olive 0.3-0.8 mL; palm 0.05-0.1 mL and peach palm 0.1-0.5 mL. The reduction in absorbance at 515 nm was measured at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 min, and then every 5 min until the reaction achieved stabilization. The choice of the wavelength is justified in the scientific methodology of determination of the antioxidant activity by the Free Radical Capture of DPPH suggested by Rufino et al. (2007).

The assays were recorded using a UV-Vis Genesis spectrophotometer (Spectronic Instruments, Rochester, NY). The data were recorded until the disappearance of DPPH in the presence of oils. The temperature was controlled at 25 °C with circulating water bath. The solvent mixture was used as reference instead of the extract. The results were obtained in triplicate and expressed as mean ± SD.

### 2.4 Kinetic modeling

The kinetic model was developed using as basis the Equations 1 to 3 to represent the radical-scavenging capacity (RSC) for the oxidation of oils. According to the governing equations and the experiments (Equations 5 and 6), the antioxidants of the oils were depleted from the medium under pseudo first-order conditions, $([R\bullet]_0 >> [OE]_0)$ following the equation:

$$\frac{d[OE]}{dt} = -k[OE] \tag{5}$$

$$[OE] = [OE]_0 \times \exp(-kt) \tag{6}$$

where: [OE] is the oil extraction concentration; $[OE]_0$ is the initial oil concentration; $k$ is the pseudofirst-order kinetic rate constant; and $t$ is the time.

The concentration of R• was calculated by mass balance using the following Equation 7:

$$[R\bullet] = [R\bullet]_0 - [OE]_0 \times \exp(-kt) \tag{7}$$

where: [R•] is the radical concentration; $[R\bullet]_0$ is the initial radical concentration; $k$ is the pseudo-first order kinetic rate constant, and $t$ is the time. [R•] concentration, herein [DPPH•] concentration, in the reaction medium was calculated according to the method of Brand-Williams et al. (1995) obtained from the calibration curve with the equation as determined by linear regression (Equation 8):

$$Abs(515nm) = 0.0137 \cdot [DPPH\bullet] - 0.029 \tag{8}$$

where [DPPH•] is expressed as μmol/L.

The pseudofirst-order kinetic rate constant ($k$) was linearly dependent on the concentration of the oil and the second-order rate constant ($k_2$) was determined by the Equation 9 (Mukai et al., 1993; Shi & Niki, 1998):

$$\frac{d[R\bullet]}{dt} = -k_2 \times [OE] \times [R\bullet] \tag{9}$$

Several oils have more than one antioxidant in their composition. In this case, Equation 10 can be written as a sum of the effect of each antioxidant:

$$\frac{d[R\bullet]}{dt} = -\sum_i k_2^i \times [OE] \times [R\bullet] \qquad (10)$$

This method for calculating the second order kinetic rate constant ($k2$) differs from the method presented by Brand-Williams et al. (1995), which determines that the concentration of R is much lower than the concentration of antioxidants ($[R\bullet]0 << [OE]0$). The assembly of the experimental data was performed using the Levenberg-Marquardt method implemented in FORTRAN 90. The second order rate constants ($k2$) were calculated to determine the RSC of the oils.

## 3 Results and discussion

The dynamics of radical scavenging showed a typical decrease in the absorption of a color change from purple to yellow, as DPPH $\bullet$ ($R\bullet$) was eliminated by anti-radicals through the hydrogen donation (Equation 3). The oxidation reaction was modeled as a second-order reaction, since two species react to form one or more oxidation products. The reaction can be recognized as a pseudo-first order reaction if one of the reactants is in excess, as assumed by Brand-Williams et al. (1995), however, to ensure greater accuracy of the model, the models developed did not make this simplification (Mukai et al., 1993; Shi & Niki, 1998; Rufino et al., 2011b).

The pseudo-first order rate constant, $k$ was linearly dependent on the initial radical scavenger concentration ($[OE]_0$). The second-order rate constants, $k_2$, which represents the rate at which R$\bullet$ can be oxidized by 1 g of oil, were calculated from Equation 9 and are presented in Table 1. This rate constant is related to the RSC present in the oils and a higher $k_2$ value corresponds to a better RSC.

Fitting of the kinetic parameters was evaluated by the goodness of fit ($R^2$) and Chi-square test. The goodness of fit for all oils was higher than 0.96 and the results from the Chi-square test showed that the model was significant at a 95% level of confidence. Thus, the kinetic model developed was able to represent the radical scavenging activity of the antioxidants present in the oils and that the model parameter could be used to analyze and compare oils.

All oils, except peach palm, have exhibited more than one kinetic period. A second kinetic period usually indicates that these oils have more than one active natural antioxidant in their composition, each one with different scavenging capacity. Antioxidants with higher CSR eliminate the R $\bullet$ radicals at a higher rate, while antioxidants with lower CSR will take longer to reduce the amount of R $\bullet$ present in the medium.

The RSC obtained for the tropical fruit oils studied herein was compared to that of olive oil, which is highly known and studied natural oil. Except for peach palm, all other oils presented a higher RSC than olive oil. The order of RSC, according to $k_2$ values (the higher $k_2$ value, the better the RSC) was nance > palm > avocado > olive > peach palm (Table 1).

Some fatty acids can act as antioxidants, as well as being sources of free radicals. This ability depends on the degree of unsaturation of fatty acids and, generally, the higher degree of unsaturation, the greater the susceptibility to oxidation. Lipids

**Table 1**. Second-order kinetic rate constants ($k_2$) for the reaction between DPPH• and the oils' antioxidant compounds.

| Oil | $k_2$ (phase 1) [1/L.min] | $k_2$ (phase 2) [1/L.min] |
|---|---|---|
| Avocado | 875.2 | 274.8 |
| Palm | 2221.3 | 1281.8 |
| Nance (var. Fortaleza) | 2696.3 | 654.3 |
| Nance (var. Pacajus) | 2488.2 | 840.4 |
| Nance (var. Paraipaba) | 2856.7 | 984.2 |
| Olive | 594.2 | 272.3 |
| Peach Palm | 11.4 | --- |

**Table 2**. Fatty acid composition of avocado, nance, olive, palm and peach palm oils.

| Fatty Acids (%) | Avocado[1] | Nance[2] | Olive[3] | Palm[4] | Peach Palm[5] |
|---|---|---|---|---|---|
| *Saturated* | | | | | |
| C16:0 Palmitic | 22.4 | 5.5 | 11.5 | 44.1 | 38.2 |
| C18:0 Stearic | 0.3 | 6.3 | 2.8 | 4.4 | 1.0 |
| *Monounsaturated* | | | | | |
| C16:1 Palmitoleic | 6.2 | -- | 0.8 | -- | 7.4 |
| C18:1 Oleic | 59.3 | 53.3 | 72.7 | 39.0 | 46.3 |
| *Polyunsaturated* | | | | | |
| C18:2 Linoleic | 10.3 | 34.9 | 8.1 | 10.6 | 6.2 |
| C18:3 Linolenic | 0.2 | -- | 0.7 | 0.4 | 1.4 |

[1]Ozdemir & Topuz (2004); [2]Rezende & Fraga (2003); [3]Ozen & Mauer (2002); [4]Gee (2007); [5]Clement et al. (1998).

belonging to the omega 3 series are less susceptible to oxidative damage than the omega 6 series because of the position of their double bonds (Richard et al., 2008).

Table 2 presents the fatty acids profile of the oils studied herein. No clear correlation between antioxidant capacity and fatty acids profile could be found. An increase in oleic acid content and in total unsaturated fatty acid content did not increase the antioxidant potential. Also, the total saturated fatty acid content was not correlated with lower radical scavenging capacity. Alves et al. (2010) evaluated the antioxidant activity in terms of the ability of the compounds present in extracts to prevent the oxidation of β-carotene, as well as to protect it from the free radicals generated during the peroxidation of linoleic acid in palm fruits native to the Amazon, where even though the concentration of the extract used was much lower for bacaba, the percentage of oxidation inhibition was high, showing the strong antioxidant capacity of this fruit in relation to the other palm species present in the study. In the same study, bacaba, tucumã and inajá presented high antioxidant capacity (92%, 92% and 80% O.I, respectively), while buriti and pupunha presented intermediate antioxidant capacity (65% and 62% O.I., respectively).

These results indicated that although the fatty acid profile may contribute to the antioxidant capacity, minor compounds in the oil composition have important effect on radical scavenging. As such, the antioxidant capacity of oils is complex and cannot be solely explained by fatty acids profile or by phenolics content.

Also it was not possible to improve the model adding information regarding fatty acid content.

In summary, the model developed to represent the radical-scavenging capacity for the oxidation of oils showed good agreement with experimental data and could be used to compare the scavenging capacity of the oils.

## 4 Conclusions

These results indicated that although the fatty acid profile may contribute to the antioxidant capacity, minor compounds in the oil composition have important effect on radical scavenging. As such, the antioxidant capacity of oils is complex and cannot be solely explained by fatty acids profile or by phenolics content. Also it was not possible to improve the model adding new information regarding fatty acid content.

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## References

Alves, C. Q., David, J. M., David, J. P., Bahia, M. V., & Aguiar, R. M. (2010). Métodos para determinação de atividade antioxidante in vitro em substratos orgânicos. *Quimica Nova*, 33(10), 2202-2210. http://dx.doi.org/10.1590/S0100-40422010001000033.

Arranz, S., Pérez-Jiménez, J., & Saura-Calixto, F. (2008). Antioxidant capacity of walnut (*Juglans regia* L.): contribution of oil and defatted matter. *European Food Research and Technology*, 227(2), 425-431. http://dx.doi.org/10.1007/s00217-007-0737-2.

Aruoma, O. I. (1998). Free radicals, oxidative stress, and antioxidants in human health and disease. *Journal of the American Oil Chemists' Society*, 75(2), 199-212. http://dx.doi.org/10.1007/s11746-998-0032-9.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenchaft und Technologie*, 28(1), 25-30. http://dx.doi.org/10.1016/S0023-6438(95)80008-5.

Bubonja-Sonje, M., Giacometti, J., & Abram, M. (2011). Antioxidant and antilisterial activity of olive oil, cocoa and rosemary extract polyphenols. *Food Chemistry*, 127(4), 1821-1827. http://dx.doi.org/10.1016/j.foodchem.2011.02.071.

Clement, C. R., Aguiar, J. P. L., & Arkcoll, D. B. (1998). Composição química do mesocarpo e do óleo de três populações de pupunha (*Bactris gasipaes*) do Rio Solimões, Amazonas, Brasil. *Revista Brasileira de Fruticultura*, 20, 115-118.

Espín, J. C., Soler-Rivas, C., Wichers, H. J., & García-Viguera, C. (2000). Anthocyaninbased natural colorants: a new source of antiradical activity for foodstuff. *Journal of Agricultural and Food Chemistry*, 48(5), 1588-1592. http://dx.doi.org/10.1021/jf9911390. PMid:10820063.

Freitas, J. B., & Naves, M. M. V. (2010). Composição química de nozes e sementes comestíveis e sua relação com a nutrição e saúde. *Revista de Nutrição*, 23(2), 269-279. http://dx.doi.org/10.1590/S1415-52732010000200010.

Gee, P. T. (2007). Analytical characteristics of crude and refined palm oil and fractions. *European Journal of Lipid Science and Technology*, 109(4), 373-379. http://dx.doi.org/10.1002/ejlt.200600264.

Miranda, C. A. S. F., Cardoso, M. G., Batista, L. R., Rodrigues, L. M. A., & Figueiredo, A. C. S. (2016). Óleos essenciais de folhas de diversas espécies: propriedades antioxidantes e antibacterianas no crescimento espécies patogênicas. *Ciência Agronômica*, 47(1), 213-220.

Mukai, K., Morimoto, H., Kikuchi, S., & Nagaoka, S. (1993). Kinetic study of free radical-scavenging action of biological hydroquinones (reduced forms of ubiquinone, vitamin K and tocopherol quinone) in solution. *Biochimica et Biophysica Acta*, 1157(2), 313-317. http://dx.doi.org/10.1016/0304-4165(93)90115-O. PMid:8323961.

Oliveira, A. C., Valentim, I. B., Goulart, M. O. F., Silva, C. A., Bechara, E. J. H., & Trevisan, M. T. S. (2009). Fontes vegetais naturais de antioxidantes. *Química Nova*, 32(3), 689-702. http://dx.doi.org/10.1590/S0100-40422009000300013.

Ozdemir, F., & Topuz, A. (2004). Changes in dry matter, oil content and fatty acids composition of avocado during harvesting time and post-harvesting ripening period. *Food Chemistry*, 86(1), 79-83. http://dx.doi.org/10.1016/j.foodchem.2003.08.012.

Ozen, B. F., & Mauer, L. J. (2002). Detection of hazelnut oil adulteration using FT-IR Spctroscopy. *Journal of Agricultural and Food Chemistry*, 50(14), 3898-3901. http://dx.doi.org/10.1021/jf0201834. PMid:12083856.

Ramalho, V. C., & Jorge, N. (2006). Antioxidantes utilizados em óleos, gorduras e alimentos gordurosos. *Quimica Nova*, 29(4), 755-760. http://dx.doi.org/10.1590/S0100-40422006000400023.

Rezende, C. M., & Fraga, S. R. G. (2003). Chemical and aroma determination of the pulp and seeds of murici (*Byrsonima crassifolia* L.). *Journal of the Brazilian Chemical Society*, 14(3), 425-428. http://dx.doi.org/10.1590/S0103-50532003000300014.

Ribeiro, E. F., & Jorge, N. (2017). Oxidative stability of soybean oil added to coffee husk extract (*Coffea arabica* L.) under accelerated storage conditions. *Food Science and Technology*, 37(spe), 5-10. http://dx.doi.org/10.1590/1678-457x.06117.

Richard, D., Kefi, K., Barbe, U., Bausero, P., & Visioli, F. (2008). Polyunsaturated fatty acids as antioxidants. *Pharmacological Research*, 57(6), 451-455. http://dx.doi.org/10.1016/j.phrs.2008.05.002. PMid:18583147.

Rufino, M. S. M., Alves, R. E., Brito, E. S., Morais, S. M., Sampaio, C. G., Pérez-Jiménez, J., & Saura-Calixto, F. D. (2007). *Metodologia científica: determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH* (Comunicado Técnico, No. 127). Fortaleza: Embrapa.

Rufino, M. S. M., Alves, R. E., Fernandes, F. A. N., & Brito, E. S. (2011b). Free radical scavenging behavior of ten exotic tropical fruits extracts. *Food Research International*, 44(7), 2072-2075. http://dx.doi.org/10.1016/j.foodres.2010.07.002.

Rufino, M. S. M., Fernandes, F., Alves, R., & Debrito, E. (2009). Free radical-scavenging behaviour of some north-east Brazilian fruits in a DPPH system. *Food Chemistry*, 114(2), 693-695. http://dx.doi.org/10.1016/j.foodchem.2008.09.098.

Rufino, M. S. M., Pérez-Jiménez, J., Arranz, S., Alves, R. E., de Brito, E. S., Oliveira, M. S. P., & Saura-Calixto, F. (2011a). Açaí (*Euterpe oleraceae*) 'BRS Pará': a tropical fruit source of antioxidant dietary fiber and high antioxidant capacity oil. *Food Research International*, 44(7), 2100-2106. http://dx.doi.org/10.1016/j.foodres.2010.09.011.

Shi, H., & Niki, E. (1998). Stoichiometric and kinetic studies on *Ginkgo biloba* extract and related antioxidants. *Lipids*, 33(4), 365-370. http://dx.doi.org/10.1007/s11745-998-0216-8. PMid:9590623.

Viana, R. D., Oliveira, F. C., Monte, M. J. S., Pereira, L. M. R., & Carvalho, J. O. (2014). Ação de antioxidantes no reaproveitamento de óleos vegetais. *Revista Interdisciplinar*, 7(4), 13-21.