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Synergistic effects of fatty acids on the performance of TBHQ in inhibiting the oxidation of corn oil

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Abstract: The synergistic effects of saturated and polyunsaturated fatty acids addition on the performance of synthetic antioxidant (TBHQ) in inhibiting the autoxidation of corn oil triacylglycerols were reported. This study utilized the effectiveness of two free fatty acids: palmitic (C16:0) and linoleic (C18:2) acids on TBHQ performance in triacylglycerols, for experimental and theoretical studies. It was shown that the TBHQ performance is depends on the concentration of fatty acids added. The addition of fatty acid decreased the triacylglycerols oxidation, thus gave less effect to the degradation of corn oil after 15 days storage time at 60 °C. The finding was supported by theoretical calculations using B3LYP hybrid functional by Gaussion09 software package, where it was found that the interaction energy is the major contributor to the performance of the TBHQ. In the presence of TBHQ, synergistic behavior was found between fatty acid and antioxidants, where complex with TBHQ were shown to be stable. Thus the addition of fatty acids either C16:0 or C18:2 may reduce the peroxide formation.

Key words: Oxidative stability, Synergistic effect, Theoretical study, TBHQ, Palmitic acid, Linoleic acid

1. Introduction

One of the most important indicators for maintaining the quality of vegetable oils is oxidative stability (Tan et al., 2002). Oxidation of lipids not only produces rancid odors and flavor but also can decrease the nutritional properties and safety in food by the formation of secondary products after cooking and processing (Frankel, 1996). The oxidative stability of fats and oils is related to their triacylglycerols (TAGs) composition and antioxidants present Hrncirik and Fritshe, 2005; Mateos et al, 2005) and iron content (Coscione and Artz, 2005).

Oxidative rancidity in vegetable oils occurs when heat, metals or other catalysts cause unsaturated oil molecules to convert to free radicals. These free radicals are easily oxidized to yield hydroperoxides and organic compounds such as aldehydes, ketones or acids (Das et al, 2009; Vlachos et al, 2006; Mannekote and Kailas, 2012), which give rise to the undesirable odors and flavors characteristics of rancid oils and fats (Naz et al, 2004). Hydroperoxides are primary products formed by oxidation mechanism is quite unstable and their decomposition products are responsible for oxidative rancidity (Azeredo et al, 2004). It rates can be decreased by addition of primary antioxidants, which compete with lipids, breaking off free radical chain.

Vegetable oil oxidation is affected by many factors including the presence of antioxidants (inhibitors) or pro-oxidants (catalyst); temperature, light, oxygen, metals and enzymes (regarding processing and storage conditions) and also unsaturated fatty acid composition and their distribution in TAG molecules (Paradiso et al, 2010; Wasowicz et al, 2004). Corn oil generally is considered to be fairly unstable to oxidation due to relatively high concentration of polyunsaturated fatty acids (PUFA). It consists of high amounts of 1,2,3-trilinoleoyl-glycerol (LLL) (25.6%) and 1,2-dilinoleoyl-3-oleoyl-glycerol (LLO) (20.5%) TAGs. It also contains considerable amounts of 1,2-dilinoleoyl-3-palmitoyl-glycerol (LLP) (16.4%), 1,2-dioleoyl-3-linoleoyl-rac-glycerol (LOO) (10.9%) and 1-palmitoyl-2-oleoyl-2-linoleoyl-rac-glycerol (POL) (10.4%), respectively (Rezanka and Rezankova, 1999). Thus, corn oil is highly susceptible to free radical oxidation reactions giving rise to the formation of lipid peroxides (Mannekote and Kailas, 2012; Valls et al, 2003) even though it was reported having better stability then soybean oil (Isabell et al, 1999).

In the vegetable oil industry, synthetic antioxidants such as TBHQ and other chelating agents are effectively being used to increase oil thermal and storage stability as well as decrease aging effect (Che Man et al, 1999; Gordon and Kouřimská, 1995; Karavalakis et al, 2011; Bera et al, 2006). Recently, study shows that the presence of

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free fatty acid (FFA) in TAGs will affect the oxidative stability, where addition of higher amount of FFA will lead to lower amounts of oxidized forms of TAGs (Paradiso et al, 2010).

A number of works have been reported on the effects of antioxidants and free fatty acids separately, on the oxidative stability of vegetable oils during processing and storage (Gordon and Kouřimská, 1995; Karavalakis et al, 2011; Bera et al, 2006). However, the available information is insufficient and most of the previous research studies involved only experimental works. There are no reports regarding theoretical studies have been carried out on the performance of antioxidant in inhibiting the autoxidation of vegetable oils in the presence of different composition of FFA in supporting the experimental works. Theoretical study, for instance using Becke's three-parameter method with Lee-Yang-Parr correlation (B3LYP) has shown to successfully predict a wide range of molecular properties (Paier et al., 2007).

Therefore, the aim of this research is to study, both theoretically and experimentally the performance of synthetic antioxidants towards oxidative stability of corn oil, in the presence or in the absence of saturated and polyunsaturated fatty acids. This experimental study is compared with theoretical calculation using Gaussian09 software. In particular, *ab initio* quantum mechanical software package Gaussian09 at the theoretical level of B3LYP/6-31G (d,p) were used for the theoretical evaluation of physical properties such as stabilization energy, dipole moment, bond index and any other physical properties that might responsible for interaction between radical and antioxidants in the presence or in the absence of fatty acid.

2. Materials and methods

2.1. Chemicals and Materials

tert-Butylhydroquinone (TBHQ) synthetic antioxidant, all pure palmitic acid (C16:0) and linoleic acid (C18:2) standards were supplied by Merck and used without any further purification. Food grade corn oil was purchased from a local grocery store. Chemicals and other solvents used for analyses such as glacial acetic acid, chloroform, potassium iodide and sodium thiosulfate were also supplied by Merck.

2.2. Experimental studies

The corn oil was mixed with fatty acids; palmitic or linoleic, at four different concentrations: 0.25, 0.5, 1.0 and 3.0 % in the absence or in the presence of TBHQ (0.5%). The mixture was heated in the oven at 60 °C for 15 days. 1 day at 60 °C is equivalent to 1 month's storage at ambient temperature (Warner et al, 1989). Samples were taken after 0, 1, 3, 6, 10, and 15 days for peroxide value (PV) analysis. PV analysis was followed AOCS method Cd 8-53 and are expressed in milliequivalents of peroxide per kilogram (meq/kg). In brief, 2.5 g of oil sample was accurately weighted in a 250 ml glass Erlenmeyer flask. Later, 15 ml solvent mixture of the acetic acid: chloroform (3:2) was added by graduated cylinder. The flask was swirled until the sample completely dissolved, followed by the addition of 0.5 ml saturated potassium iodide solution. Distilled water was added (30 ml) and shake vigorously to liberate the iodine from the chloroform layer. 1 ml of starch solution was added as an indicator using 1 ml pipette. 0.05 M sodium thiosulfate filled in the burette and the sample has been titrated until the blue gray color disappears in the aqueous (upper layer). The volume of sodium thiosulfate used was recorded to the two decimal places. Blank titration was carried out under the same conditions. The process was repeated for three times for all samples to avoid the errors that may occurred during titration.

2.3. Theoretical studies and DFT/B3LYP optimization

In this research, methyl linoleate (ML) C9OO• was used to represent corn oil. All quantum mechanical calculations were performed using ab initio quantum mechanical software package of Gaussian09 (Gaussian09. Revision D.01.Gaussian, Inc., Wallingford CT) at the theoretical level of DFT/B3LYP with 6-31G (d,p) basis sets and GaussView 5.0 (GaussView. Version 5. Semichem Inc., Shawnee Mission KS) molecular visualization program package to study the physical interaction between ML C9OO• with antioxidants, ML C9OO• with fatty acids and ML C9OO• with antioxidants and fatty acids, during the oxidation processes.

The molecule was first optimized to the minimum potential energy. In this study, TBHQ, fatty acids (palmitic and linoleic acids) and also combination of antioxidants and fatty acids were forced to form complexes with $C_9OO^•$. The optimization produced the physical parameter such as SCF energy, molecular geometry and dipole moment. The following step was NBO calculation which evaluate:(i) the intermolecular distance between $C_9OO^•$ with O-H of antioxidants and fatty acids, (ii) bond length, bond dissociation energy and also bond index of O-H antioxidants and fatty acids. The data collected were analyzed and ranked according to their efficiency.

3. Results and discussion

In this research, we have applied DFT/B3LYP to study the structure, molecular and the physical properties of the fatty acid-oil-antioxidant complexes in order to relate the synergistic effect of saturated and polyunsaturated fatty acids on the TBHQ performance in inhibiting the oxidation of corn oil.

In brief, corn oil will be deteriorated during the storage at 60 °C for 15 days, as demonstrated by their PV value. PV measures the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. For this purpose, five samples including pure corn oil (CO), corn oil with 0.5% TBHQ and 0.25% palmitic acid (CO + TBHQ 0.5% + PA 0.25%), corn oil with 0.5% TBHQ and 0.5% palmitic acid (CO + TBHQ 0.5% + PA 0.5%), corn oil with 0.5% TBHQ and 1.0% palmitic acid (CO + TBHQ 0.5% + PA 1.0%), corn oil with 0.5% TBHQ and 3.0% palmitic acid (CO + TBHQ 0.5% + PA 3.0%), and corn oil with 0.5% TBHQ alone without fatty acid (CO + TBHQ 0.5%) were subjected to PV measurement during the 15 days of storage at 60 °C (Fig. 1).

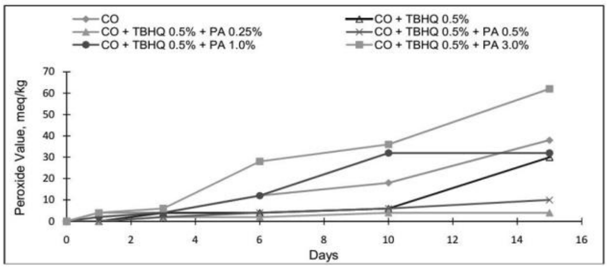

Fig. 1: Samples subjected to PV measurement during the 15 days of storage at 60 °C

One day at 60°C is equivalent to one month's storage at ambient temperature (Warner et al, 1989). Based on the results obtained, the PV increased with the increasing of storage time (Fig. 1). PV is a measure of the concentration of peroxides and hydroperoxides formed in the initial state (Zhang et al, 2010). The first 3 days, show a relatively low PV for all samples before suddenly increased toward the 15 days. Due to the high levels of unsaturation, corn oil is highly susceptible to free radical oxidative reactions, giving rise to the formation of peroxide over the period of time (Valls et al, 2003). Result shows that the PV for corn oil without TBHQ (CO) will gradually increase during the storage. The addition of TBHQ 0.5% can prevent the oxidative deterioration of corn oil by reducing the decomposition of oil TAGs. Additionally, experimental data shows that the addition of less than 0.5% w/w PA improved the performance of TBHQ 0.5% w/w in reducing the peroxide formation of corn oil after 16 days of storage. However, the addition of more than 1.0% PA increased the oxidation of corn oil, showing further deterioration of oil TAGs. The increasing trend in PV during storage was slow at the first 3 days and then a sharp increase was observed toward the end of storage time.

A number of researchers have reported that TBHQ is a good antioxidant in minimizing the oxidation activity of oils (Che Man et al, 1999; Dunn et al, 2005; Pimpa et al, 2009), However there are limited knowledge to relate this with their physical parameters associated with molecular geometry, interaction and bond dissociation energies. Our theoretical calculation using B3LYP hybrid functional revealed that the combination of $C_9OO^{\bullet}$ and TBHQ has high stabilization energy (-31.68 kJ/mol) as stated in the Table 1. Thus, suggest that the higher value of stabilization energy of the complex is related to the compatibility between the two species, which results to having longer half-life. Moreover, the addition of TBHQ can improve the oxidative stability of oil because of the hydroxyl (-OH) group of the antioxidant is very active. The hydrogen is abstracted from –OH and donated to the oxidized free radical to inhibit the rate of oxidation in oil (De Guzman et al, 2009; Loh et al, 2006). The theoretical calculation showed that, TBHQ easily donate the -H radical to hydroperoxyl/radicals. From the bond index analyses, we also found that the antioxidant have similar performance to donate their -H radical (Table 1).

Table 1: Total electronic energy and interaction energy of C9OO•, antioxidants, fatty acids and their complexes

System	SCF Energy, a.u	Interaction energy, kJ/mol	Different between the original complex, kJ/mol
C9OO• + TBHQ	-1584.678424	-31.6783	
C9OO• + TBHQ + PA	-2364.215760	-67.0857	
C9OO• + TBHQ	-1584.682288	-41.8214	-10.1431
C9OO• + PA	-1824.240029	-25.8914	17.9149
C9OO• + TBHQ + LA	-2440.376453	-67.4584	
C9OO• + TBHQ	-1584.682221	-41.6473	-9.9690
C9OO• + LA	-1900.400634	-26.0321	18.6089

For an antioxidant to efficiently quench all destructive radical intermediates, it would have to be lower than that of the peroxyl and hydroperoxyl radicals (Fox and Stachowiak, 2007).

The effect of fatty acid unsaturation addition was tested by changing the oil sample with saturated C16:0 PA (Fig. 1) to the oil samples with C18:2 linoleic fatty acid (LA) (Fig. 2). Fig. 2 shows the PV performance of pure corn oil and corn oil with TBHQ 0.5% w/w in the presence of different percentage of LA. It clearly showed that the addition of up to 1.0% w/w LA managed to reduce the formation of peroxide, hence gave lower PV to corn oil. Eventhough this result slightly contradict to the previously reported where low amounts of added FFA caused a further increase of the levels of oxidized TAG pointing out pro-oxidant activities (Paradiso et al, 2010), our theoretical calculation show significant finding that this effect might be related to the synergistic behavior between TBHQ 0.5% and additional fatty acid (Table 1).

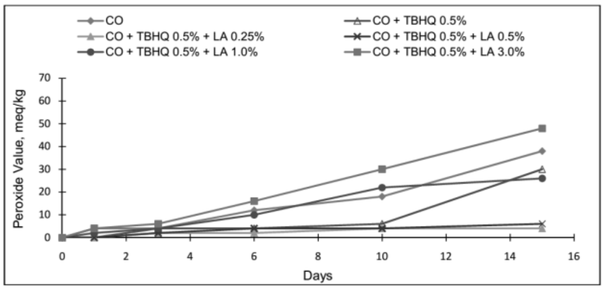

Fig. 2: The effect of fatty acid unsaturation addition

Theoretical calculation demonstrated that addition of both fatty acids have similar effect on the performance of TBHQ in the corn oil sample (Table 1). The presence of fatty acid either saturated (PA) or polyunsaturated (LA), will increase the interaction energy between $C_9OO^\bullet$ and TBHQ. For instance, the combination of $C_9OO^\bullet$ + TBHQ + LA formed more stable complex compared to the ones without fatty acid (Fig. 3). Fig. 3 showed the most stable molecular structure for the complex with particular self-consistent-field (SCF) energy recorded as -2440.376453 a.u. The similar effect of both fatty acids due to their similar interaction energy in the tri-species systems: $C_9OO^\bullet$ + TBHQ 0.5 + LA (-67.46 kJ/mol) is almost similar to the tri-species of $C_9OO^\bullet$ + TBHQ 0.5 + PA (-67.09 kJ/mol). Results showed that, the slightly higher interaction energy in the presence of LA gives more stability to this complex, compare to PA (Fig. 4) with SCF energy -2364.215760 a.u. Based on the theoretical results as tabulated in Table 1, the formation of the peroxides in corn oil (Figs. 1 and 2) are expected to reduce after adding either one of the fatty acids, in the presence of TBHQ. We found that, for instance, CO + TBHQ without fatty acid having peroxide value of 25 meq/kg with interaction energy of -31.6783 kJ/mol, while the addition of LA will decreased the peroxide value to less than 5meq/kg with interaction energy of -67.4584 kJ/mol (Fig. 2 and Table 1), hence increased the storage life of oil. Theoretical study also revealed that the interaction energy is the major contributor to the performance of the antioxidants, compare to other physical parameters using the B3LYP hybrid functional theory.

In the conclusion, the presence of lower amount of fatty acids either saturated (PA) or polyunsaturated (LA) will improve the performance of TBHQ in inhibiting the oxidation, thus greatly reduced the formation of peroxide of corn oil. The addition of TBHQ 0.5% can prevent the oxidative deterioration of corn oil by reducing the decomposition of oil TAGs. In the presence of TBHQ 0.5%, the addition of 0.25% w/w up to 1.0% w/w LA reduced the decomposition of TAGs of corn oil, hence increased the storage life of corn oil. However, only up to 0.5% PA addition able to inhibit the oxidation of oil. There exists a synergistic behavior between fatty acids, PA and LA, with the other two constituents of the matrix, corn oil and TBHQ. These experimental observations were supported by theoretical studies where complex with LA (I_{LA}= - 67.46 kJ/mol) is more stable than the complex with PA (I_{PA} = - 67.09 kJ/mol), where in the addition of more than PA 1.0% increased the decomposition of TAGs of corn oil and decreased the performance of TBHQ.

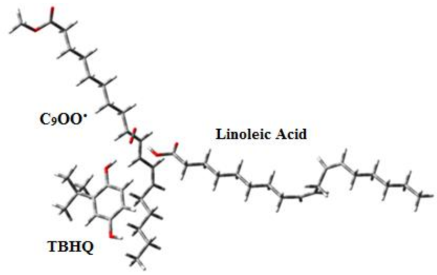

Fig. 3: More stable complex

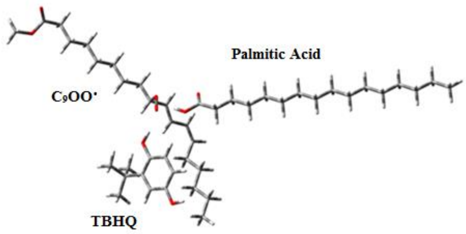

Fig. 4: Higher interaction energy in the presence of LA and more stability to the complex, compare to PA

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