AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

# Effect of storage conditions on rancidity and antioxidant activity of gac oil compared with healthy oils

Montip Chamchong[a,*], Darun Waeruwanaruk[a,†], Chanikarn Guntornkun[a,†], Tania Alam[b]

[a] *Department of Food Engineering, Faculty of Engineering at Kamphaengsaen, Kasetsart University, Nakhon Pathom 73140, Thailand*

[b] *Department of Nutrition and Food Engineering, Faculty of Allied Health Science, Daffodil International University, Dhaka 1207, Bangladesh*

**Article Info**

**Article history:**
Received 29 November 2019
Revised 18 January 2021
Accepted 27 January 2021
Available online 9 April 2021

**Keywords:**
Antioxidant,
Gac oil,
Kinetic,
Lycopene,
Rancidity

**Abstract**

The kinetic changes were studied of the chemical attributes (oxidative and hydrolytic rancidity, lycopene, beta-carotene, antioxidant capacity) of gac oil during storage with and without limited oxygen at different temperatures. The results were compared with those of other healthy oils (virgin olive, virgin rice bran, virgin camellia tea seed) stored under the same conditions. Samples (each 7 mL) of the oils were stored in 10 mL open-capped and closed-capped amber glass bottles and kept at four different storage temperatures (5°C, 25°C, 40°C, 60°C) for 1 mth. Before being kept in storage, the gac oil had the highest antioxidant capacity (mean ± SD; 143.53 ± 10.62 mg ascorbic acid equivalents (AAE)/g oil) and after storage at 40–60°C, its degradation rate of antioxidant capacity was the lowest. The antioxidant capacity of the gac oil remained the highest in the range 46.75 ± 1.05 mg AAE/g oil to 54.06 ± 0.29 mg AAE/g oil despite decolorization from dark-red to an orange-yellow color at these storage temperatures (40–60°C). The rate constant of reaction regarding hydrolytic rancidity for rice bran oil increased more rapidly than for the other oils when the storage temperature increased.

## Introduction

Gac oil, obtained mostly from the aril part of the gac fruit, *Momordica cochinchinensis* (Cucurbitaceae), is a healthy oil that is considered to have very high antioxidant capacity and very high amounts of lycopene and beta-carotene (Kha et al., 2013). The gac oil contains a high amount of unsaturated fatty acid (UFA) of up to 69%, of which 35% is monounsaturated fatty acid (MUFA) according to Vuong (2000). Many researchers have reported that the aril from fully ripe gac fruit, the peel color of which is red-orange had a lycopene content at least five times higher than other well-known fruits and vegetables such as grapefruit, tomato, papaya, guava and watermelon (Aoki et al., 2002) while the beta-carotene content was about eight times higher than in carrot (Vuong, 2000; Aoki et al., 2002; Rao and Rao, 2007). In addition, Müller-Maatsch et al. (2017) reported that the carotenoids (lycopene, beta-carotene) from gac fruit aril were more bioaccessible than from carrot root and tomato fruit.

Other healthy oils include camellia (*Camellia oleifera*) tea seed oil, which has been accepted as healthy product in Thailand but is not as popular as other healthy oils. This type of oil produced from other species (*Camellia sinensis*) has long been consumed in China and Japan as a tea drink as well as for cooking oil (Chen, 2007). Because of its medical therapy and composition, this tea seed oil (from both species) has been recognized as the "olive oil from the east" (Sahari and Amooi, 2013). Camellia tea seed oil has such good quality and low amount of fat that is well accepted that it is not inferior to genuine virgin olive oil (Robards et al., 2009). Some researchers reported that virgin tea seed oil and olive oil have a UFA content as high as

† Equal contribution.

* Corresponding author.
E-mail address: fengmoc@ku.ac.th (M. Chamchong)



https://doi.org/10.34044/j.anres.2021.55.2.06

87% while the MUFA content is up to 76% and 69%, respectively (Orsavova et al., 2015; Weng et al., 2018). Although scientific evidence is relatively limited, rice bran oil (RBO) is tenaciously believed to be a healthy vegetable oil in Asian countries due to specific components such as gamma-oryzanol and tocotrienols that could participate in its hypocholesterolemic effects (Sugano et al., 1999). The UFA content in virgin rice bran oil is about 78% of which 44% are MUFA (Orsavova et al., 2015).

For antioxidant activity assessment in food products, many researchers have applied more than one method at a time and usually used other food samples for comparison or as a benchmark, since each assay or method has its own procedure which is suitable for different major physicochemical properties of food. For example, Pellegrini et al. (2003) studied the total antioxidant capacity in six types of oils using 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay. It was found that soybean oil with tocopherol as a food additive for rancidity protection had the highest amount of antioxidant capacity. The second best was corn oil followed by sunflower oil, cold-pressed olive oil (extra virgin olive oil), olive oil and the lowest was peanut oil.

However, there has been the only limited publication of available reports or research papers on oil deterioration due to rancidity or anti-oxidation degradation in healthy oils such as gac aril oil compared with other healthy oils after storage that have been manufactured and in the market for a long time. Therefore, to maximize benefit, research is needed to collect information on the factors affecting such qualities of this oil as well. The current research aimed to study kinetic changes in the rancidity and the antioxidant activity of gac aril oil during storage under conditions of limited and unlimited oxygen at various temperatures. The results were compared with other commercial types of healthy oils (extra virgin olive oil, virgin rice bran oil, virgin camellia tea seed oil) kept under the same conditions.

## Materials and Methods

### *Experimental design*

The experiment used a completely randomized factorial design with four factors: four types of oils (gac oil, camellia tea seed oil, rice bran oil, olive oil), two conditions (limited and unlimited oxygen), four levels of storage temperature (5°C, 25°C, 40°C, 60°C) and 10 storage sampling periods (0 d, 2 d, 4 d, 6 d, 8 d, 10 d, 12 d, 14 d, 19 d, 24 d, 30 d).

### Materials and oil sample preparation

Gac fruits at the fully ripe stage were purchased from a community enterprise in Baan Plak Mai Lai, Thung Kwang subdistrict, Kamphaengsaen district, Nakorn Pathom province, Thailand. The aril separation and pretreatment for mechanical oil extraction followed the method of Bhumsaidon and Chamchong (2016). Freshly cold-pressed extracted *Camellia oleifera* tea seed oil (virgin oil) without other treatments was provided by the factory of the Royal Phatra Pat Project while commercial, freshly cold-pressed rice bran oil was purchased directly from the manufacturer (the cooperative of Organic Agricultural Surin Rice Fund Limited). However, only the olive oil (extra virgin grade, Healthy Mate™ manufactured by JOE&CO. SRL, Italy) which could not be produced in Thailand, was purchased as an imported product from a modern trade store.

Before use, the four kinds of oil were centrifuged at 10,000 revolutions per minute (rpm) for 10 min to remove moisture or any insoluble solids. Then, 7 mL of each oil was sampled and these separate samples were put in closed-capped and open-capped amber bottles and then separated for storage at four different temperatures (5°C, 25°C, 40°C, 60°C) for 1 mth. The storage conditions for all samples aimed to have minimal exposure to light so that all bottles were placed in corrugated cardboard boxes before leaving the storage room at 5°C and 25°C, bot at 40 ± 2% relative humidity (RH) but without the corrugated cardboard boxes when leaving a hot-air oven at 40°C and 60°C in a laboratory room (30±2°C ambient temperature and 59 ± 2% RH). Chemical attributes (oxidative and hydrolytic rancidity, lycopene and beta-carotene contents, antioxidant capacity) were measured at days 0, 2, 4, 6, 8, 10, 12, 14, 19, 24 and 30 of storage. Each treatment was duplicated.

### *Chemical reagents*

Chemicals for rancidity were purchased from Merck KGaA (Germany). Acetone, hexane and methanol used for lycopene and beta-carotene analysis and in antioxidant assay were analytical grade purchased from Daejung Chemicals & Metals (Korea). Reagents for antioxidant assay consisted of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) purchased from Sigma-Aldrich® (USA) and L (+) -ascorbic acid, AR grade obtained from Daejung Chemicals & Metals (Korea).

### *Analysis of chemical attributes*

The peroxide value (PV) and acid value (AV) were determined using the titration method described by the standard procedures 2.501 and 2.201 (International Union of Pure and Applied Chemistry-IUPAC, 1993) for oxidative and hydrolytic rancidity, respectively. The degree of hydrolytic rancidity quantified as the AV was related to the amount of free fatty acid in the oil. Oxidative rancidity is caused by an autoxidation reaction, the target site of which is on the double bonds of unsaturated fatty acid together with oxygen from the air (Malekian, 2000). As a result, a peroxide linkage is formed at the α-methylene (-CH=CH-) site from this type of fat and chemical analysis for oxidative rancidity is carried out by quantifying the hydroperoxide and is known as the PV (Kaleem et al., 2015).

The lycopene and beta-carotene contents were quantified using the method of Nagata and Yamashita (1992). First, the carotenoids were extracted from 1 mL of oil using 10 mL of mixed solution (acetone:hexane at 4:6 volume per volume (v/v)) in a high-speed homogenizer (IKA, T 25 Digital ULTRA-TURRAX, Germany) at 15,000 rpm for 10 min to enhance miscibility. The supernatant extracted solution was measured for absorbance at 453 nm ($A_{453}$), 505

nm ($A_{505}$), 645 nm ($A_{645}$)and 663 nm ($A_{663}$) using a ultraviolet-visible spectrophotometer (UV-1800; Shimadzu; Japan). The lycopene and beta-carotene contents were calculated using Equations 1 and 2:

$$\text{Lycopene} = -0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453} \quad (1)$$

$$\beta\text{-carotene} = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453} \quad (2)$$

The result was converted into milligrams of lycopene or $\beta$-carotene from the original 100 mL of oil.

Modified DPPH assay using ascorbic acid as a standard solution was applied to analyze the antioxidant capacity in the camellia tea seed oil, rice bran oil and olive oil but with some additional modifications for the gac oil. Since all the oils would not dissolve very well with 0.1 mM of DPPH in methanol as solvent, the premix of each oil and hexane at the ratio of 1:10 (v/v) according to Burits and Bucar (2000) was prepared first and then 0.05 mL of this oil mixture was used as an oil sample which was added in 5 mL of 0.1 mM DPPH solution. After being mixed well using a vortex mixer and kept in the dark at room temperature for 30 min, the light absorption was measured at 540 nm (Liu et al., 2008). The data were collected as $A_S$ in Equation 3. Then, 5 mL of DPPH solution mixed with 0.05 mL hexane (without adding oil) was used as the oil sample and the absorption was recorded as $A_c$. A solution of 5 mL methanol and 0.05 mL hexane was used as a blank for all cases. However, the gac oil originally was dark red-yellowish in color, and after being prepared with a mixture of hexane (1:10 v/v), it still had an orange-yellowish color that might cause interference in the interpretation of the absorption of light during the measurement, since the amount of antioxidant capacity determined using this method depends on the fading color from deep purple of DPPH to light yellow. Therefore, another solution ($A'_s$) of 5 mL methanol mixed with 0.05 mL of gac oil was used to offset the light-yellow color using Equation 3 (Panjasutaros, 2017). The percentage DPPH activity was expressed as in Equation 3 and further calculation was required to convert to units of milligrams ascorbic acid equivalents per gram of oil (AAE/g oil). All chemical analyses were done in triplicate.

$$\%DPPH\ inhibition = \frac{(A_C - A_S - A'_S)}{A_C} \times 100 \quad (3)$$

*Kinetic assessment*

The kinetic changes of the chemical attributes of each oil during storage were investigated using Equation 4. The proper degree of reaction rate was determined from all treatments for each amount of chemical attributes (c) based on the minimal standard error of estimation (SEE) as in Equation 5 between predicted and experimental values. The rate constant (k) of each oil for each chemical attribute in Equation 6 was used for the quality comparison:

$$r = \frac{dc}{dt} = kc^m \quad (4)$$

$$SEE\ of\ c = \sqrt{\frac{\sum_{n=1}^{n}(c_{prediction} - c_{experiment})^2}{n-1}} \quad (5)$$

$$k = k_0 e^{-\frac{E_a}{RT}} \quad (6)$$

where m is the degree of the reaction rate (r), $k_0$ is the constant in the Arrhenius equation of the change in the quality attribute during storage of each oil and does not depend on temperature.

Several papers have reported on kinetic degradation of the chemical attributes in a food model system under different storage conditions (Henry et al., 1998; Lee and Chen, 2002; Ax et al., 2003).

Statistical analysis

The effects of the studied factors were investigated using analysis of variance and sample means were compared using Duncan's new multiple range test in the R software package, version 3.3.2 (R Core Team, 2017). Significance was tested at the $p < 0.05$ level. Values were presented as mean ± SD.

## Results and Discussion

*Initial value of rancidity and antioxidant capacity of each oil*

From Table 1, gac oil had the lowest initial PV (1.36 ± 0.75 milliequivalents (meg)/kg oil) of oxidative rancidity before storage in this experiment, while those of rice bran and olive oil were significantly higher (9.54 ± 0.52 meg/kg oil and 9.37 ± 1.01 meg/kg oil, respectively). These results were probably because of the lowest value of unsaturated fatty acid was in gac oil (69%, Vuong, 2000) compared to camellia tea seed (87%; Weng et al., 2018), olive oil (87%; Orsavova et al., 2015) and rice bran oil (87%; Orsavova et al., 2015). This could result in greater potential for oxidative reaction with oxygen for the latter three oil types. However, the camellia tea seed and the olive oil had rather similar percentages of unsaturated and monounsaturated fatty acids (Orsavova et al., 2015; Weng et al., 2018) but their PVs were quite different. The much lower PV of camellia tea seed oil was probably because it was freshly extracted from the food factory while the olive oil was an imported product and therefore had been processed at a much earlier time and perhaps was not as fresh.

**Table 1** Initial values (mean ± SD of two replications) for rancidity, antioxidant capacity and fatty acid content of each oil at day 0 (before storage)

| Oil | PV (meq/kg oil) | Moisture content (%wb) | AV (mg KOH/g oil) | DPPH activity (mg AAE/g oil) |
|---|---|---|---|---|
| Gac aril | 1.36 ± 0.75$^b$ | 0.17 ± 0.03$^a$ | 1.06 ± 0.08$^b$ | 143.53 ±10.62$^a$ |
| Tea seed | 3.27 ± 1.99$^b$ | 0.10 ± 0.02$^b$ | 1.25 ± 0.20$^b$ | 80.30 ± 3.86$^c$ |
| Olive | 9.37 ± 1.01$^a$ | 0.10 ± 0.01$^b$ | 1.06 ± 0.08$^b$ | 79.02 ± 1.04$^c$ |
| Rice bran | 9.54 ± 0.52$^a$ | 0.16 ± 0.02$^a$ | 4.20 ± 0.20$^a$ | 111.44 ± 7.27$^b$ |

PV = peroxide value; AV = acid value; DPPH = 2, 2-diphenyl-1-picrylhydrazyl; meq = milliequivalents; wb = wet basis; AAE = ascorbic acid equivalents
Mean ± SD values in a column with different lowercase superscripts indicate significant ($p < 0.05$) differences.

In the storage study, the initial PVs of olive and rice bran oil were quite high or almost not acceptable for virgin oil quality (>15 meq/kg oil; CODEX STAN, 1981). However, in most cases in the country where it is not in a cultivated area with a Mediterranean climate, virgin olive oil is available for purchasing is an imported product. Thus, degradation of this oil has already commenced before it becomes available in the markets of importing countries. Likewise for virgin rice bran oil, the rice bran raw material itself tends to get rancid more quickly before being used for oil extraction (Malekian, 2000). Virgin rice bran oil is still used mostly as food supplement packing in a soft gel capsule or as a raw material of cosmeceutical/ nutraceutical products. Therefore, it was still meaningful to use them for comparison.

The initial AVs of the gac, camellia tea seed and olive oils were very low (about 1 mg KOH/g oil) and not significantly different. Only rice bran oil had a very high value (4.20 mg KOH/g oil), even though its moisture content was as low as for the gac oil. However, this type of oil was extracted from rice brans which contain a high amount of endogenous lipase that could lead to rapid hydrolytic rancidity in its virgin oil (Malekian, 2000). Thus, it was most likely to have the highest AV among the other oils.

Considering that a high moisture content in the oil might affect its quality in terms of being prone to rapid rancidity, all these oils still had initial moisture contents below the regulated level (0.2% wet basis; CODEX STAN, 1981) for edible oils and fats. In addition, the initial PVs and AVs of all these virgin oils were below the regulated levels (15 meg/kg oil, 4 mg KOH/g oil, respectively).

The antioxidant capacity was determined based on the initial value of the DPPH activity of all types of oils before storage. Gac oil had the highest value of DPPH activity (143.53 ± 10.62 mg AAE/g oil), while the rice bran, camellia tea seed and olive oil had lower values (111.44 ± 7.27 mg AAE/g oil, 80.30 ± 3.86 mg AAE/g oil and 79.02 ± 1.04 mg AAE/g oil, respectively). The highest value of antioxidant capacity in the gac oil was probably due to its high lycopene and beta-carotene contents (502.93 ± 9.73 mg/100 mL oil and 793.70 ± 6.00 mg/100 mL oil, respectively; Table 3) which could perform as free radical scavengers. The rice bran oil had the second-best activity due to the performance of tocopherol/tocotrienol or vitamin E and oryzanol, which are inherent in the oil, while there was no significant difference between the values for the camellia tea seed oil and olive oil. However, the highest values of monounsaturated fatty acid in the camellia tea seed and olive oil probably indicated a lower risk of oxidative rancidity compared to the gac aril and rice bran oils.

*Kinetics assessment during storage*

From Table 1, the initial values of rancidity based on the antioxidant capacity of each oil at day 0 varied in broad ranges. Therefore, to investigate the change in these attributes, was considered more appropriate to perform a kinetic study and compare the degradation rate constant of each oil.

*Oxidative rancidity*

The kinetic study graphed the PVs during the 30 d of storage at various temperatures. It was found that the oxidative rancidity reaction (PV) had a zero order rate. From Fig. 1A, the rate constant (k) of this reaction for camellia tea seed oil kept in a closed-capped bottle with a limited oxygen supply at a storage temperature of 5–40°C had the lowest value compared to the rest of the oils. However, when the storage temperature increased in the range 40–60°C, the PV for the camellia tea seed oil was significantly higher than for the other three types of oils, indicating that at low temperatures, tea seed oil had the lowest oxidative rancidity since this oil had the highest percentage of monounsaturated fatty acid (76%; Weng et al., 2018). Therefore, of the total unsaturated fatty acid contained in the camellia tea seed oil, the content of polyunsaturated fatty acids was only minor (about 11%; Weng et al., 2018). Oxidative rancidity at the polyunsaturated fatty acid bonds was less likely. However, when the temperature rose over a long storage period, the rancidity reaction of the camellia tea seed oil accelerated more rapidly than in the other three types of oils, probably due to the rapid decomposition of the active substances of α-tocopherol, beta-carotene and some total phenols that could retard the oxidation reaction. This assumption could be supported by the disappearance of the yellowish color of the tea seed oil that faded away until it was clear or colorless like coconut oil when being kept with minimal exposure to the light in an open-capped bottle or it became a pale yellowish color in a closed-capped bottle after storage at 60°C for only 14 d, as shown in Fig. 2A. As a result, the DPPH activity of camellia tea seed oil kept in the dark at 60°C for 30 d (Table 2) was the lowest (9–10%) compared to the other three types of oils for both closed-capped and open-capped bottles.

There was a noticeable change in the reddish color of the gac oil. When the experiment was carried out in closed-capped amber bottles at 60°C for 14 d, the reddish color of gac aril oil did not change, as shown in Fig. 2B. However, when kept in the dark in an open-capped amber bottle under the same conditions, the color became pale orange as shown in Fig. 2B. This indicated a reduction in the amounts of some active substances in the gac oil that could prevent an oxidation reaction, such as lycopene and beta-carotene, which affected the reddish color in the oil. The rate constant, k, of the oxidative rancidity of gac oil, was highest for oil being kept in an open-capped amber bottle at 5–40°C (Fig. 1B) due to having the lowest percentage of monounsaturated fatty acid. However, when the storage temperature was higher (40–60°C), the k value of the oxidative rancidity rate of gac oil in the closed-capped bottles was lowest, probably due to the highest percentage of antioxidant remaining as indicated by the DPPH activity (38–39% in Table 2).

For the olive and rice bran oils, even though their initial PVs were quite high, the k values of this reaction (Figs. 1A and 1B) were low and similar, indicating that the hydroperoxide product of oxidative rancidity was low throughout the study period for both closed-capped and open-capped bottles. This could be explained by the fact that olive oil contained high amounts of monounsaturated fatty acids (as high as in camellia tea seed oil). The unsaturated degree of oil had an impact on its oxidative stability. In addition, this oil appears to be particularly

rich in strong antioxidant substances such as vitamin E (α-tocopherol), carotenoids and phenolic compounds consisting of simple phenols such as hydroxytyrosol and complex phenols such as oleuropein (Oliveras-López et al., 2014). Virgin rice bran oil also contained high amounts of tocopherol/tocotrienol or vitamin E and oryzanol, which are good antioxidants that still work well in this temperature range (5–60°C). Antioxidants can reduce the autogeneration of peroxides, delaying the onset of oxidation and rancidity (Boskou et al., 2006). Consequently, their oxidative rancidity reactions were slow. Another interpretation of the lower k values is that the secondary change of primary oxidative product (hydroperoxide) decomposition into various secondary products of oxidation including aldehydes, ketones and hydrocarbons, occurred faster than the primary change of hydroperoxide formation from unsaturated fatty acids. Thus, off-flavor or undesirable flavor of odor-active compounds from these two types of oils were higher than the others.Thus, since the initial PVs of each oil did not start within the same level (low PV)—olive oil and rice bran oil were quite high, almost exceeding the recommended values or the quality index for product shelf life—the kinetic study would be an appropriate tool to assess the product degradation due to the increasing rate of PV during storage. These two kinetic study cases for olive oil and rice bran oil indicated that oil having a higher initial PV does not necessarily have a higher final PV.

*Hydrolytic rancidity*

In the study of kinetic changes, hydrolytic rancidity was studied similarly to oxidative rancidity. Under the experimental conditions, the reaction of hydrolytic rancidity had a zero order rate for all four types of oils. The reaction rate constant (k) varied between 5°C and 60°C (Figs. 1C and 1D) with rice bran oil having a high value of k and increasing most rapidly when the temperature increased or possibly because it had the greatest hydrolytic rancidity. This was probably caused by the high level of endogenous lipase enzyme in virgin rice bran oil (Malekian, 2000). In particular, in the open-capped or unlimited oxygenated oil sample, this reaction would be more rapid. For example, the k value of rice bran oil kept in an open-capped bottle stored at 60°C was as high as 0.1434 mg KOH/(g oil·day), whereas that kept in a closed-capped bottle was lower at 0.1347 mg KOH/(g oil·day). However, for tea seed oil, the k value was very low and changed very little. In addition, that of olive oil was also low and also showed almost no change. These results were because both oils used in this experiment contained significantly lower moisture percentages compared to the gac and rice bran oil. Thus, hydrolytic rancidity was less due to less moisture being available to break down fat into glycerol and its component fatty acids with the association of heat and enzymes. Nevertheless, it was noticeable that gac oil had a higher rate constant (k) than rice bran oil at low temperature (5°C), probably due to the interference of the strong indigenous reddish color of gac oil to the phenolphthalein indicator color at the endpoint of the reaction during chemical analysis. However, when the temperature was increased, the rice bran oil increased this type of rancidity much more rapidly than the other three types of oils.

*Antioxidant capacity*

When the four types of oils were stored at four different temperature levels in the range 5–60°C for 30 d, the antioxidant capacity (indicated as the DPPH activity) decreased for all types of oils, as shown by the rate constant (k) of the reduction rate of DPPH activity (Figs. 1E and 1F) which was achieved under a first order reaction, where a higher k value represented a more rpaid rate of decline. A higher storage temperature tended to have more effect on chemical composition degradation, which decreased its antioxidant capacity. When kept in open- or closed-capped amber bottles, the tea seed oil had the highest reducing rate or lowest DPPH activity, followed by olive oil. For the gac and rice bran oils, the k values of antioxidant capacity diminishing were very low compared with the other two types of oils. However, at a low storage temperature of 5–25°C and in a closed-capped bottle, gac oil had a higher degradation rate of antioxidant capacity than rice bran oil, probably because the bioactive compounds, which were the source of antioxidant capacity in the gac oil, decomposed more than in the rice bran oil. When the gac oil was kept in an open-capped bottle, the decomposition rate was more rapid. This could be observed from the fading of the reddish color as in Fig. 2B while the colors of rice bran and olive oil were still yellowish or showed almost no change.

Therefore, the active substances available in camellia tea seed oil for both closed- and open-capped bottles (namely the polyphenolic compound types, carotenoid (beta-carotene) and α-tocopherol), seemed to be depleted most rapidly compared to the other three types of oils, such as the lycopene and beta-carotene in the gac oil and the tocopherol/tocotrienol and oryzanol in the rice bran oil. The degradation rate of olive oil ranked the second highest. Even though it had a similar fatty acid content to the camellia tea seed oil, the active substances had different levels of performance. The main antioxidants in this extra virgin olive oil should be the lipophilic and hydrophilic phenols, especially tocopherols and tocotrienols, with the presence of a small amount of carotenoids (Servili et al., 2014). In this regard, over 90% of the tocopherols were α-tocopherol, the concentration of which is characterized by strong variation depending on pedoclimatic factors and agronomic practices, such as the area of origin, the cultivar and the stage of fruit ripening (Inglese et al., 2011). On the other hand, the rice bran oil was the most stable, considering its DPPH activity and the percentage remaining after storage (Table 2). It was found that the conditions of the being closed or open or supply of oxygen being limited or unlimited did not have significant effects on the DPPH activity in each oil after storage at 5–60°C for 30 d. Therefore, the rice bran oil in a closed-capped bottle stored at 5°C had the highest percentage of remaining DPPH activity (71%). In contrast, the camellia tea seed oil had DPPH activity that remained as minimal percentages at storage temperatures in the range 5–60°C.

*Lycopene and beta-carotene contents*

From Table 3, the lycopene and beta-carotene contents in freshly extracted gac oil initially were as high as 502.9±9.73 mg/100mL oil and 793.7±5.97 mg/100 ml oil, respectively. This was in agreement with reported data from Bhumsaidon and Chamchong (2016), while the lycopene and beta-carotene contents were much lower in the

tea seed, rice oil and olive oil. It should be noted that there was no beta-carotene found in olive oil even though many research papers reported that some antioxidant activity might be due to the presence of carotenoids (Mínguez-Mosquera et al., 1990; Rahmani and Csallany, 1991) as well as polyphenols or vitamin E. The carotenoid contents reported are related to the analytical method used (Boskou et al., 2006). After storage at 60°C for 30 d, the lycopene content in gac oil was reduced to 136.18±10.53 mg/100mL oil and 142.36±16.39 mg/100mL oil in closed-capped and open-capped bottles, respectively. Even though these amounts were not significantly different, the color of gac oil in the open-capped amber bottle had clearly faded from dark-red to orange-yellow over the 14 d of storage, while that in the closed-capped bottle still remained reddish (Fig. 2B). This meant the lycopene and beta-carotene which are associated with the dark reddish color of gac oil degraded much more rapidly in the open-capped bottles than in the closed ones in early storage. However, finally, after long storage (30 days) at 60°C, the lycopene and beta-carotene in the closed-capped and open-capped bottles degraded further without any significant difference (Table 3) because both of these phytonutrients are highly susceptible to light, heat and oxygen (Ax et al., 2013). The decolorization (orange-yellow) of gac oil might have been due to isomerization together with degradation of lycopene. Ax et al. (2013) found that 9-cis lycopene was the most labile isomer in the oil-in-water emulsion system. Increasing the temperature (90°C) without exposure to oxygen could lead to a significant decrease in the concentrations of all-trans and 13-cis isomers accompanied by a concurrent increase in the 9-cis isomer concentration. Therefore, in the current research, after long storage (30 days at 60°C) one part of all-trans and 13-cis isomers in open-capped bottles of gac oil might be affected by thermal degradation, resulting in a reduction in total lycopene content but another part would be under isomerization due to oxidative degradation increasing the 9-cis isomer. Therefore, the lycopene contents in both open-capped and closed-capped bottles were lower but not significantly different.

*Change in lycopene and beta-carotene during storage*

The first order rates of kinetic change of lycopene and beta-carotene during storage for 30 d in each oil (Figs. 1G–1J) were the lowest in the gac oil, which had the highest amount of both carotenoids initially with the lowest degradation rate, while in the other three types of oils the degradation rates were comparable. When the temperature increased from 5°C to 60°C, the lycopene degradation rates of all oil samples in the closed-capped bottles linearly increased only slightly. However, with the open-capped bottles, the degradation rates of lycopene in the rice bran and olive oils reduced with increasing temperature. These phenomena were probably due to the initial availability of very small amounts of lycopene in both types of oils, with much more rapid depletion of the lycopene occurring at the higher storage temperatures, resulting in a very small amount of lycopene left at the 30 d evaluation of the rate constant, k.

For beta-carotene, the camellia tea oil had the highest rate of degradation when it was kept in a closed-capped bottle and this rate increased much more rapidly than that of the other three types of oils. As mentioned earlier the available beta-carotene could retard the oxidation reaction, therefore its degradation rate was high. The rice bran oil had the second worst performance in a closed-capped bottle. However, when the rice bran oil was kept in an open-capped bottle or with an unlimited supply of oxygen, the degradation rate was higher than that in closed-capped bottles and increased much more rapidly, especially at a high temperature and was the highest when the storage temperature was in the range 40–60°C. This indicated that oxygen had more impact on the degradation rate of beta-carotene in rice bran oil than for the other types of oils since this oil had far more rapid hydrolysis rancidity.

**Table 2** Mean DPPH activity ± SD (milligrams of g ascorbic acid equivalents per gram of oil) for antioxidant capacity of each oil on day 30

| T (°C) | Oil type | Condition | DPPH activity | Remaining (%) | T (°C) | Oil type | Condition | DPPH activity | Remaining (%) |
|---|---|---|---|---|---|---|---|---|---|
| 5 | Tea seed | Closed | $10.32 \pm 5.23^{fg}$ | 14.4 | 25 | Tea seed | Closed | $8.31 \pm 0.34^{g}$ | 11.6 |
| | | Open | $11.12 \pm 2.73^{fg}$ | 15.6 | | | Open | $7.34 \pm 1.02^{g}$ | 10.3 |
| | Olive | Closed | $22.20 \pm 1.04^{e}$ | 30.5 | | Olive | Closed | $18.45 \pm 2.37^{ef}$ | 25.4 |
| | | Open | $21.00 \pm 0.09^{e}$ | 28.9 | | | Open | $18.11 \pm 6.83^{ef}$ | 24.9 |
| | Rice bran | Closed | $72.81 \pm 12.40^{ab}$ | 71.2 | | Rice bran | Closed | $53.34 \pm 11.03^{c}$ | 52.2 |
| | | Open | $60.42 \pm 9.33^{ab}$ | 59.1 | | | Open | $39.19 \pm 0.11^{c}$ | 38.4 |
| | Gac | Closed | $75.10 \pm 1.15^{a}$ | 54.4 | | Gac | Closed | $71.99 \pm 0.77^{b}$ | 52.1 |
| | | Open | $74.76 \pm 0.29^{a}$ | 54.1 | | | Open | $61.30 \pm 11.29^{b}$ | 44.4 |
| 40 | Tea seed | Closed | $7.10 \pm 0.00^{g}$ | 9.9 | 60 | Tea seed | Closed | $7.26 \pm 0.00^{g}$ | 10.2 |
| | | Open | $10.48 \pm 5.46^{g}$ | 14.7 | | | Open | $6.46 \pm 0.23^{g}$ | 9.0 |
| | Olive | Closed | $17.64 \pm 5.79^{ef}$ | 24.3 | | Olive | Closed | $15.50 \pm 1.42^{fg}$ | 21.3 |
| | | Open | $16.57 \pm 9.96^{ef}$ | 22.8 | | | Open | $12.28 \pm 0.09^{fg}$ | 16.9 |
| | Rice bran | Closed | $38.71 \pm 0.11^{d}$ | 37.9 | | Rice bran | Closed | $37.18 \pm 0.23^{d}$ | 36.4 |
| | | Open | $39.19 \pm 1.25^{d}$ | 38.4 | | | Open | $37.02 \pm 0.23^{d}$ | 36.2 |
| | Gac | Closed | $54.06 \pm 0.29^{c}$ | 39.2 | | Gac | Closed | $52.50 \pm 1.34^{c}$ | 38.0 |
| | | Open | $53.79 \pm 2.77^{c}$ | 39.0 | | | Open | $46.75 \pm 1.05^{c}$ | 33.9 |

DPPH = 2, 2-diphenyl-1-picrylhydrazyl

Mean ± SD values in a column with different lowercase superscripts indicate significant ($p < 0.05$) differences.

**Table 3** Mean lycopene and beta-carotene contents ± SD before storage and on day 30 of the storage period in open-capped and closed-capped amber bottles

| T (°C) | Oil type | Condition | Initial lycopene (mg/100mL oil) | Lycopene after storage (mg/100mL oil) | Initial beta- carotene (mg/100mL oil) | Beta-carotene after storage (mg/100mL oil) |
|---|---|---|---|---|---|---|
| 60 | Tea seed | Closed | 55.00 ± 9.54$^{b}$ | 5.36 ± 0.18$^{b}$ | 122.27 ± 0.67$^{b}$ | 9.92 ± 3.16$^{b}$ |
| | | Open | | 6.22 ± 0.10$^{b}$ | | 9.31 ± 1.91$^{b}$ |
| | Olive | Closed | 14.57 ± 5.49$^{c}$ | 2.27 ± 1.83$^{b}$ | ND | ND |
| | | Open | | 4.24 ± 0.21$^{b}$ | | ND |
| | Rice bran | Closed | 31.91 ± 13.09$^{bc}$ | 5.28 ± 0.99$^{b}$ | 41.15 ± 35.84$^{c}$ | 5.12 ± 0.65$^{b}$ |
| | | Open | | 9.24 ± 0.00$^{b}$ | | 4.15 ± 5.87$^{b}$ |
| | Gac | Closed | 502.93 ± 9.73$^{a}$ | 136.18 ± 10.53$^{a}$ | 793.70 ± 6.00$^{a}$ | 301.29 ± 51.28$^{a}$ |
| | | Open | | 142.36 ± 16.39$^{a}$ | | 273.81 ± 8.88$^{a}$ |

ND = not detected

Mean ± SD values in a column with different lowercase superscripts indicate significant ($p < 0.05$) differences.

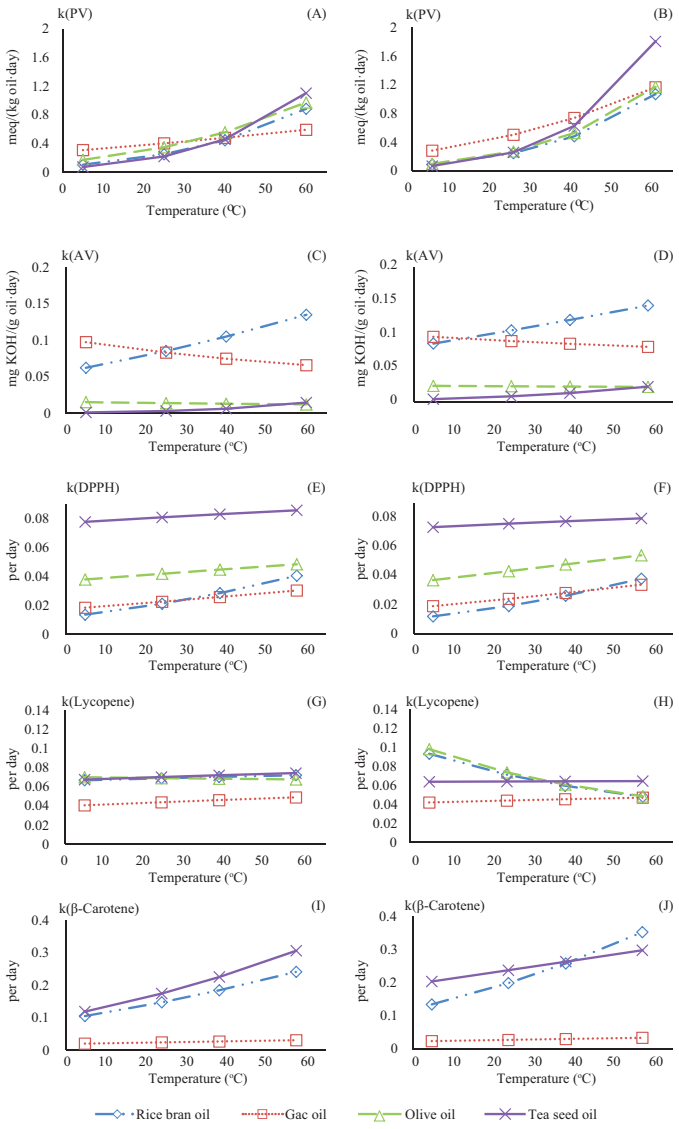


**Fig. 1** Rate constant of reaction (k) changing with temperature from 5°C to 60°C for each oil in closed-capped bottles (on the left side) and in open-capped bottles (on the right side): (A) and (B) peroxide value (PV); (C) and (D) acid value (AV); (E) and (F) 2, 2-diphenyl-1-picrylhydrazyl (DPPH); (G) and (H) lycopene; (I) and (J) *β*-carotene, where *β*-carotene was not detectable in olive oil using the described method

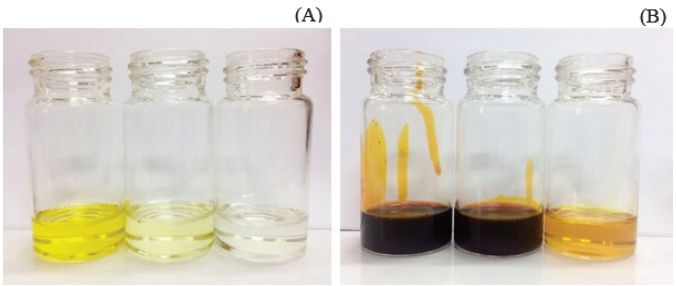


**Fig. 2** Change in oil color under different storage conditionsfor before storage (left), 14 d with closed-capped at 60°C (middle), 14 d with open-capped at 60°C (right): (A) tea seed oil; (B) gac oil

## Conflict of Interest

The authors declare that there are no conflicts of interest.

## Acknowledgements

The research was funded by the Faculty of Engineering at Kamphaengsaen, Kasetsart University, Nakhon Pathom, Thailand.

## References

Aoki, H., Kieu, N.T.M., Kuze, N., Tomisaka, K., Chuyen, N.V. 2002. Carotenoid pigments in gac fruit (*Momordica cochinchinensis* Spreng). Biosci. Biotechnol. Biochem. 66: 2479–2482. doi: 10.1271/bbb.66.2479.

Ax, K., Mayer-Miebach, E., Link, B., Schuchmann, H., Schubert, H. 2003. Stability of lycopene in oil-in-water emulsions. Eng. Life Sci. 3: 199–201. doi.org/10.1002/ elsc.200390028

Bhumsaidon, A., Chamchong, M. 2016. Variation of lycopene and beta-carotene contents after harvesting of gac fruit and its prediction. Agr. Nat. Resour. 50: 257–263. doi.org/10.1016/j.anres.2016.04.003

Boskou, D., Blekas, G., Tsimidou, M. 2006. Olive oil composition. In: Boskou, D. (Ed.). Olive Oil. AOCS Press. Urbana, IL, USA, pp. 41–72. doi: 10.1201/9781439832028.pt2

Burits, M., Bucar, F. 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res. 14: 323–328. doi.org/10.1002/1099-1573(200008)14: 5<323::AID- PTR621>3.0.CO;2-Q

Chen, Y.H. 2007. Physiochemical properties and bioactivities of tea seed (*Camellia oleifera*) oil. M.Sc. thesis, Department of Food, Nutrition and Packaging Sciences, Clemson University. Clemson, SC, USA.

CODEX STAN. 1981. Codex standard 19-1981 for edible fats and oils not covered by individual standards, Amendment: 2009, 2013 and 2015. Food and Agriculture Organization of the United Nations. Rome, Italy.

Henry, L.K., Catignani, G.L., Schwartz, S.J. 1998. Oxidative degradation kinetics of lycopene, lutein and 9-cis and all-trans *β*-carotene. J. Am. Oil Chem. Soc. 75: 823–829. doi.org/10.1007/s11746-998-0232-3

Inglese, P., Famiani, F., Galvano, F., Servili, M., Esposto, S., Urbani, S. 2011. Factors affecting extra-virgin olive oil composition. In: Janik, J. (Ed.). Horticultural Reviews. John Wiley & Sons Pubs. Hoboken, NJ, USA, pp. 83–148.

International Union of Pure and Applied Chemistry -IUPAC. 1993. A Guide to IUPAC Nomenclature of Organic Compounds. Blackwell Scientific Publications, Ltd. Oxford, UK.

Kaleem, A., Aziz, S., Iqtedar, M., Abdullah, R., Aftab, M., Rashid, F., Shakoori, F.R., Naz, S. 2015. Investigating changes and effect of peroxide values in cooking oils subject to light and heat. FUUAST J. Biol. 5: 191–196.

Kha, T.C., Nguyen, M.H., Roach, P.D., Parks, S.E., Stathopoulos, C. 2013. Gac fruit: Nutrient and phytochemical composition, and options for processing. Food Rev. Int. 29: 92–106. doi.org/10.1080/87559129.2012.692141

Lee, M.T., Chen, B.H. 2002. Stability of lycopene during heating and illumination in a model system. Food Chem. 78: 425–432. doi.org/10.1016/S0308-8146(02)00146-2

Liu, D., Shi, J., Colina Ibarra, A., Kakuda, Y., Xue, S.J. 2008. The scavenging capacity and synergistic effects of lycopene, vitamin E, vitamin C, and *β*-carotene mixtures on the DPPH free radical. LWT - Food Sci. Tech. 41: 1344–1349. doi.org/10.1016/ j.lwt.2007.08.001

Malekian, F. 2000. Lipase and Lipoxygenase Activity, Functionality, and Nutrient Losses in Rice Bran During Storage. LSU Agricultural Experiment Station Reports. Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center. Baton Rouge, LA, USA.

Mínguez-Mosquera, M., Gandul-Rojas, B., Garrido-Fernández, J., Gallardo-Guerrero, L. 1990. Pigments present in virgin olive oil. J. Am. Oil Chem. Soc. 67: 192–196.

Müller-Maatsch, J., Sprenger, J., Hempel, J., Florence Kreiser, F., Carle, R., Schweiggert, R.M. 2017. Carotenoids from gac fruit aril (*Momordica cochinchinensis* [Lour.] Spreng.) are more bioaccessible than those from carrot root and tomato fruit. Food Res. Int. 99: 928–935. doi.org/10.1016/j.foodres.2016.10.053

Nagata, M., Yamashita, I. 1992. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. J. Jpn. Soc. Food Sci. 39: 925–928. doi.org/10.3136/nskkk1962.39.925

Oliveras-López, M.J., Berná, G., Jurado-Ruiz, E., Serrana, H.L.G.D.L, Martín, F. 2014. Consumption of extra-virgin olive oil rich in phenolic compounds has beneficial antioxidant effects in healthy human adults. J. Funct. Foods 10: 475–484.

Orsavova, J., Misurcova, L., Ambrozova, J.V., Vicha, R., Mlcek, J. 2015. Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. Int. J. Mol. Sci. 16: 12871–12890. doi.org/10.3390/ijms160612871

Panjasutaros, C. 2017. The study of stabilized emulsion of gac oil for water based emulsion of fortified food product. M.Eng. thesis, Faculty of Engineering at Kamphaengsaen, Kasetsart University. Nakhon Pathom, Thailand.

Pellegrini, N., Serafini, M., Colombi, B., Rio, D.D., Salvatore, S., Bianchi, M., Brighenti, F. 2003. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. J. Nutr. 133: 2812–2819. doi.org/10.1093/jn/133.9.2812

Rahmani, M., Csallany, A. 1991. Chlorophyll and β-carotene pigments in moroccan virgin olive oils measured by high-performance liquid chromatography. J. Am. Oil Chem. Soc. 68: 672–674. doi.org/10.1007/BF02662293

Rao, A.V., Rao, L.G. 2007. Carotenoids and human health. Pharmacol. Res. 55: 207–216. doi.org/10.1016/j.phrs.2007.01.012

Robards, K., Prenzler, P., Ryan, D., Zhong, H. 2009. Camellia oil and tea oil. In: Moreau, R.A., Kamal-Eldin, A. (Eds.). Gourmet and Health-Promoting Specialty Oils. AOCS Press. Urbana, IL, USA, pp. 313–343.

Sahari, M.A., Amooi, M. 2013. Tea seed oil: Extraction, compositions, applications, functional and antioxidant properties. Acad. J. Med. Plants 4: 68–79. doi: 10.13140/RG.2.1.3147.8644

Servili, M., Sordini, B., Esposto, S., Urbani, S., Veneziani, G., Maio, I.D., Selvaggini, R., Taticchi, A. 2014. Biological activities of phenolic compounds of extra virgin olive oil. Antioxidants 3: 1–23. doi.org/10.3390/antiox3010001

Sugano, M., Koba, K., Tsuji, E. 1999. Health benefits of rice bran oil. Anticancer Res. 19: 3651–3657.

Vuong, L.T. 2000. Underutilized *β*-carotene-rich crops of Vietnam. Food Nutr. Bull. 21: 173–181. doi.org/10.1177/156482650002100211

Weng, X., Yun, Z., Zhang, C. 2018. Comparison of the characteristics of two kinds of tea seed oils: Oil-tea seed oil and green-tea seed oil. J. Food Stud. 7: 56–67. doi.org/10.5296/jfs.v7i1.12289