A New Approach for Stabilization of Gac Oil by Natural Antioxidants

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Abstract

Gac oil contains a high amount of unsaturated fatty acids and carotenoids that are easily degraded during production and storage. Therefore, it is necessary to have appropriate methods to limit the oxidation in gac oil that leads to undesirable by-products. Green tea and rosemary extracts exhibit excellent antioxidant activities against oxidation in vegetable oils. Here, the protective effects of green tea and rosemary extracts on gac oil were evaluated for 7 consecutive days at 60ºC. Supplementation with 4000 mg/kg green tea and rosemary extracts significantly improved oil stability by reduction of 35 and 47% peroxide values; produced higher carotenoid content of about 0.31 and 0.35 mg/g; and maintaining 82 and 92% rancimat values when compared to initial samples, respectively. In comparison to BHA and BHT at 150 mg/kg, green tea extracts at 4000 mg/kg showed higher antioxidant efficacies and sensory scores.

Keywords: gac oilantioxidants; carotenoids; peroxide value; rancimat
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1. Introduction

Gac fruit (*Momordica cochinchinensis* Spreng) is widely used in traditional Asian cuisines due to its notable bright red seed aril, which is rich in β-carotene and lycopene [1]. Gac aril contains high concentration of fatty acid (22% w/w), including oleic (29%), palmitic (32%) and linoleic acids (20%) [2]. Moreover, the concentrations of β-carotene and lycopene are found in gac oil at 2.6 and 2.4 mg/g, respectively [3]. In addition, it also has a high level of vitamin E (at 330 mg/kg) which is considered to be one of the natural antioxidants that protects gac oil from oxidation [4]. Carotenoids are considered to be beneficial in decreasing the risk of certain cancers and eye diseases [5]. Gac supplements are a good source of provitamin A and and the consumption of gac products

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such as “xoi gac” may be particularly beneficial to children suffering from anemia [6]. In addition, carotenoids such as α- and β-carotene can be metabolized into vitamin A and have an important role related to chronic disease prevention [7]. However, gac oil easily undergoes oxidation processes during storage and food processing that lead to nutrition degradation and unexpected rancidity [3, 8, 9] due to the high content of carotenoids and unsaturated fatty acid (about 70% of total fatty acid in the aril and half of them are polyunsaturated) [10]. Therefore, the use of antioxidants can be considered as a solution to help limit the degradation of gac oil during storage and processing.

Synthetic antioxidants, particularly butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been widely used to stabilize oils and fat due to their high antioxidant capacity and outstanding performance in the attenuation of oxidation reactions [11-13]. However, these antioxidants are considered to have adverse effects on human health [14, 15]. Consequently, replacing these synthetic antioxidants with natural antioxidants has become a focus of interest for researchers. Recently, many efforts have been made to prevent the oxidative degradation of oils through the use of natural antioxidant compounds [16, 17]. Natural antioxidants not only improve the stabilization of edible oils but they also increase the nutraceutical value of the oils. Such antioxidants are supposed to be safe and promptly accepted by consumers [18]. It has been reported that natural plant extracts (NPE) including green tea, rosemary, grapes and berries are potential antioxidants [11, 14, 16, 19-23].

Green tea and rosemary extract are widely used in fat oxidation prevention. Green tea extract contains important antioxidants including catechin, epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate and other flavonoids [24]. The effect of green tea extract on the oxidation of seal blubber oil and menhaden oil under Schaal oven test at 65°C was reported. The excellent antioxidant activity of green tea extract in oil samples was demonstrated and its effectiveness was higher than those of BHA and BHT at concentrations greater than 200 ppm [25]. Green tea extract can be used to decelerate the oxidative process of fats in sponge cake products [26].

Rosemary extract is considered to be a safe type of natural antioxidant composed of carnosol and carnosic acid, the two frequently studied components for antioxidant activity. In addition, rosmarinic acid is another important antioxidant in rosemary and has been extensively studied in other plants [27]. According to Erkan *et al*. [28], rosemary extract, when compared to blackseed essential oil, had the higher phenolic content resulting in a higher antioxidant activity. Besides, many edible oils are effectively protected against several oxidative reactions when added to rosemary extract [11, 24, 29-32]. However, there have been no studies using natural antioxidants for gac oil. Gac oil is an abundant source of bio-accessible carotenoids (lycopene and β-carotene), which quickly degrade during storage. In this study, we evaluated the antioxidant efficacy of rosemary extract and green tea extract on gac oil for the first time, and compared their effectiveness with some synthetic antioxidants. These samples were assessed through peroxide value, carotenoids content and rancimat index.

2. Materials and Methods

2.1 Materials

A commercial rosemary extract was obtained from the leaves of *Rosmarinus officinalis* and it had a rosmarinic acid concentration of 60 mg/g. The green tea used was a flavoring preparation containing naturally occurring polyphenol content of 90 mg/g as gallic acid equivalent. Both the green tea and rosemary extract were provided by Vitablend Netherland. BHA and BHT were obtained from Golden Hope Nha Be edible oil company. Other chemicals including acetic acid (99.5%), potassium

dichromate (99.8%), petroleum ether (99.99%), chloroform (99.99%), sodium thiosulfate (99.99%), and potassium iodide (99.5%) were purchased from Chanu Co., Ltd., Viet Nam.

2.2 Gac oil extraction

Gac fruits were supplied by retailers in Ho Chi Minh city, Viet Nam. The fruit samples were immediately processed and gac arils were collected. Pulp, peel and seeds were discarded. The gac arils were then dried in a microwave at 630 W for 65 min [33]. A laboratory hydraulic press machine with pressures of 175 kg/cm² was used to extract the oil from the arils and the recovery oil was 90%. Finally, gac oil was deposited and filtered before storing at 4°C until analysis.

2.3 Sample preparation

In previous studies, green tea and rosemary extract at concentration of 2500-5000 mg/kg provided the best protection [34, 35]. The gac oil samples were heated to 50°C before adding antioxidants. The antioxidants were added and stirred so as to completely dissolved them in the oil samples and the stirring was continuously maintained at 55°C for 10 min. Then, the natural antioxidants (rosemary extracts, green tea extract) were added to the pre-heated gac oil samples at concentrations of 1000 and 4000 mg/kg (R1: 1000 mg/kg rosemary extract; R4: 4000 mg/kg rosemary extract; G1: 1000 mg/kg green tea extract; G4: 4000 mg/kg green tea extract).

Synthetic antioxidants (BHA and BHT) were employed at their legal limit of 150 mg/kg. According to Food and Drug Administration (FDA 2001) and European Union (Council Directive 1995), the safe usage levels range from 100 to 200 mg/kg [36-39]. The control samples (M0) had none of the antioxidants added. All the investigated samples and control samples were placed in reagent bottles and stored in an oven at 60°C [40-42]. The analyses were carried out after every 24 h.

2.4 Analytical methods

2.4.1 Peroxide value (PV)

The PV of gac oil samples was determined according to AOAC Official Method 965.33 [43]. Gac oil (5g) was placed into 250 ml Erlenmeyer flask and mixed with 30 ml of a mixture of acetic acid and chloroform (3:2, v/v). Then, 0.5 ml of saturated potassium iodide solution was added. After 1 min, 30 ml of distilled water was added and the titration process was started using sodium thiosulfate 0.01 N with a starch solution as an indicator until the solution became colorless. The results were calculated as milliequivalents of active oxygen per kg (meq/kg) of oil sample as follows:

$$\mathrm{PV(meq/kg)} = \frac{(\mathrm{S} - \mathrm{B}) \times \mathrm{N} \times 1000}{\text{Mass of sample (g)}} \quad (1)$$

S (ml): The titration amounts of 0.01 N sodium thiosulfate for the sample
B (ml): The titration amounts of 0.01 N sodium thiosulfate for blank
N: The normality of sodium thiosulfate solution.

2.4.2 Determination of carotenoid content

Total carotenoids were examined using the method described by Cenkowski *et al*. [44]. The method is based on the solubility of carotenoid in organic solvent. As carotenoids dissolve in organic

solvent, a yellow color is produced, and the color intensity of the solution is directly proportional to the content of carotenoid. Each oil sample (0.1 g) was accurately weighed in a 100 ml Becher. Then 10 ml of hexane was added to dissolve the oil. The color of the solution was measured at 460 nm by spectrophotometer and hexane was used as a blank. The standard curve was formulated by dissolving 0.1 g of β-carotene in 100 ml hexane and diluting to five different concentrations using two-fold serial dilution. The carotenoid concentrations were interpolated from the standard curve and expressed as mg/g β-carotene equivalents.

2.4.3 Antioxidant activity index

The Rancimat apparatus (Metrohom Series 679) was used to evaluate the oxidation stability of oil by determining the induction time of the treated and control oil samples. Oil samples (2.5 g) were weighed in reaction vessels and preheated for 10 min. Air (20 l/h) was bubbled through oil and temperature was adjusted to 100ºC. Reaction vessels were connected with absorption vessels containing 60 ml of deionized water via teflon tubing. During the heating of the sample with air, volatile compounds were collected in deionized water in the absorption vessels and this led to increasing water conductivity. Here, measuring electrodes were immersed in water, ensuring that they were continually measuring and recording the conductivity. The time from induction period until oil starts to become rancid is called induction period. The time taken to reach the conductivity induction times was recorded [45]. The antioxidant activity of supplemented antioxidants was expressed as a protection factor (PF), which is the ratio of induction time of the sample with and without antioxidant [46].

2.4.4 Sensory Evaluation

The samples were sensitively evaluated by 10 candidates selected from final year students who had been trained by the Faculty of Food Science and Technology, Food Industry University. Aroma, taste ad overall acceptability were assessed via a 9-point hedonic scale, the scale values of which ranged from 9 for extremely good and 1 for unacceptable [47].

2.5 Statistical analyses

The experiments were repeated three times. The results were expressed as the average of three replicates ± standard deviation. The results were analyzed by one-way ANOVA followed by the T-test using statgraphics software (Statgraphics Technologies, Inc., USA) and observed differences were considered statistically significant when $p < 0.05$.

3. Results and Discussion

3.1 The antioxidant performances of rosemary and green tea extracts

3.1.1 Peroxide values

The changes in PV of gac oil samples treated with rosemary and green tea extracts at different concentrations in 7 days are presented in Figure 1.

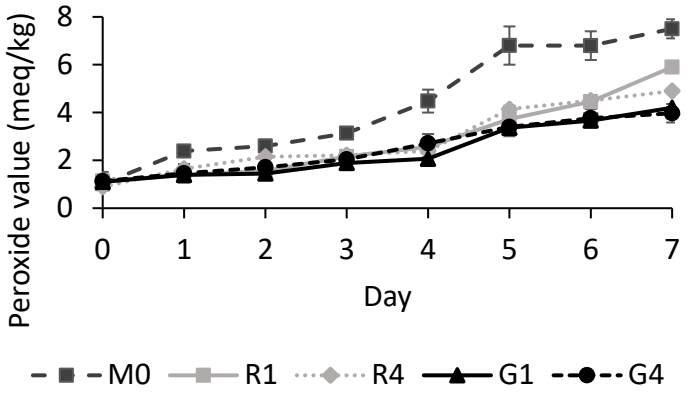

Figure 1. Peroxide value of sample treated with 1000 and 4000 mg/kg rosemary or green tea extracts for 7 days at 60ºC. M0: control sample; R1: 1000 mg/kg rosemary extract; R4: 4000 mg/kg rosemary extract; G1: 1000 mg/kg green tea extract; G4: 4000 mg/kg green tea extract

Regarding the control sample (without antioxidant), the highest PV was reached after five days of incubation (no significant PV among day 5, 6 and 7 ($p < 0.05$)). The samples treated with rosemary and green tea extracts showed significant effectiveness in reducing PV during the storage period time. Particularly, in comparison with control samples (Figure 1), the treatments with 1000 mg/kg rosemary and green tea extracts resulted in decreases of PV of 45 and 51% on day 5; and of 21 and 44% on day 7, respectively. Similar, reductions of 39 and 50% on day 5; and 35 and 47% on day 7 also observed for the supplementation of 4000 mg/kg rosemary and green tea extracts.

The oil oxidation process produces peroxides as the main initial products. Therefore, the higher the PV of the oil sample indicated, the lower the chemical stability [48]. Besides, high temperature and light are two common peroxide forming agents [49]. The peroxide value of all samples generally increased continuously over the whole 7 days of testing due to the formation of free radicals during heat treatment. These results were consistent with those obtained in previous studies [20, 40]. Malheiro *et al*. [50] studied the formation of hydroperoxide during microwave processing and its ability to prevent the formation of peroxide in the presence of tea extract.

According to Pokorny *et al*. [51], antioxidants can inhibit or retard oxidation by scavenging free radicals acting as primary antioxidant, or by a mechanism that does not involve direct scavenging of free radicals, where they act as secondary antioxidant. Based on our results, green tea extracts produced a significant reduction of peroxide value in gac oil. Green tea is notable for its phenolic compounds with antioxidant effects [52]. It is known that hydroxyl groups from aromatic rings of polyphenols react with lipid-free radicals thereby retarding lipid oxidation. Besides, polyphenols have the ability to scavenge oxygen radicals by giving an electron for the free radical in the electron transfer mechanism [53]. Green tea extract had an impressive antioxidant effect on oil stability and inhibition of hydroperoxides formation [54]. Besides, the effect of rosemary extract depends on the antioxidant activity of the extract, which is known as the phenolic content [28]. It was reported that phenolic compounds in rosemary extract can prevent transition metal ions from changing; for example, they can inhibit the change of Fe^{2+} ion into Fe^{3+} ion. Furthermore, transition metal ions at low valent states react rapidly with hydroperoxide to form alkoxy radicals in the propagation of lipid peroxidation, thus reducing induction period [55]. Comparing the effectiveness of two types of antioxidants, oil treatment with green tea extract showed greater attenuation of PV than oil treated with rosemary extract at the same concentration [22].

3.1.2 Carotenoids content

The effects of rosemary and green tea extracts on carotenoids content for 7 days at 60°C on gac oil are shown in Figure 2. In this study, the carotenoids content in the original gac oil was 2.3 ± 0.3 mg/g while in the previous study of Tran and Dang [56], about 2.0 mg/g carotenoids obtained from aril. The levels of carotenoids depend on the season, cultivar condition, harvest time, maturity and storage conditions [3].

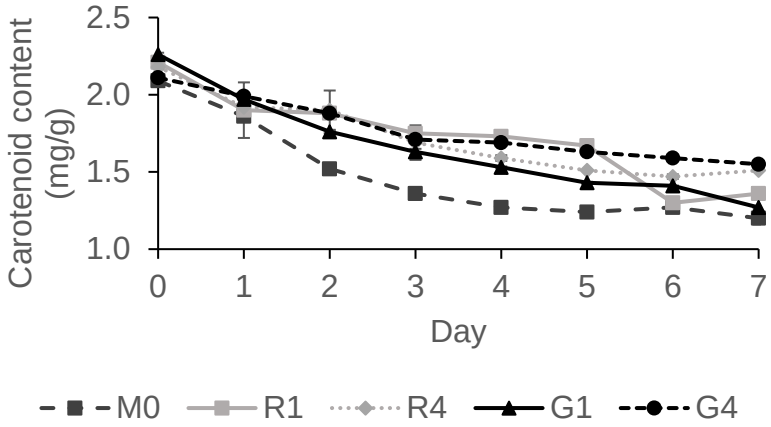

Figure 2. Carotenoids content of samples treated with 1000 and 4000 mg/kg rosemary or green tea extracts for 7 days at 60°C. M0: control sample; R1: 1000 mg/kg rosemary extract; R4: 4000 mg/kg rosemary extract; G1: 1000 mg/kg green tea extract; G4: 4000 mg/kg green tea extract

The carotenoids content predominantly decreased during the 7 days storage at 60°C may be due to thermal decomposition of carotenoids. As Mercadante [57] reported, carotenoids are easy to undergo degradation process that are induced by high temperature, low pH, light and reactive spices because they contain several conjugated double bonds. Many studies showed that heat and oxygen promote isomerization into *cis*-carotenoids which are less stable and more susceptible during storage [58-60]. Moreover, the loss of carotenoids is supposed to be related to decomposition reactions accelerated by temperature [58]. Previous work indicated that β-carotene was degraded in the first few hours of heat treatment [61]. The longer the incubation time, the more carotenoids were degraded [58, 61, 62]. Moreover, the thermal decomposition of carotenoids is dependent on the concentration of antioxidants [63]. Besides, the carotenoid contents of R1 and R4 slightly increased on day 7, compared with those observed on day 6. This was probably due to the high-temperature and long-time treatment causing the changes in oil color of these samples. Consequently, it possibly affects the carotenoid content measured by spectrophotometer at 460 nm. In addition, hydroperoxides in polyunsaturated oils may lead to formation of peroxyl or alkoxyl radial that react with β-carotene and the antioxidants protected β-carotene from degradation [62].

However, the samples supplemented by rosemary and green tea extract presented notably higher carotenoids concentrations compared to control sample in the last days of storage period. Particularly, for with 4000 mg/kg rosemary and green tea extract treatment, the carotenoids contents were higher than control by about 0.31 and 0.35 mg/g, respectively, at the day 7 of storage. Our results suggest the protective capability of natural antioxidants for carotenoids in gac oil at all testing concentrations. The higher concentrations (4000 mg/kg) of rosemary extract and green tea extract

showed higher carotenoid remaining, especially in the case of prolonging incubation time. According to Misharina and Kiseleva [63], the efficiency of inhibition of carotenoid autoxidation depended in a complex way on the concentration of antioxidants. Rosemary extract inhibited autoxidation in proportion to its concentration. In addition, rosemary extract and green tea extract also illustrated the effective inhibitory effects on β-carotene changes in buffalo homogenates lipid [64].

3.1.3 Rancimat value

Rancimat test is considered to be a rapid, economic, easy to handle and reproducible method that provides direct evidence for the tendency against oxidative rancidity of oil [65]. Therefore, it can be used as an effective and quick tool to assess the quality of fats and oils [66-68]. The longer induction times reflect the higher resistance to oxidation or good efficiency of the supplementing antioxidants [69]. The effect of rosemary and green tea extract on maintaining rancimat value was illustrated in Figure 3.

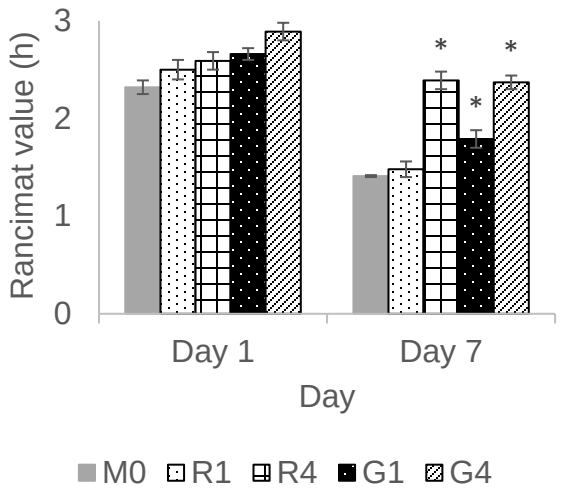

Figure 3. Rancimat values of samples treated with 1000 and 4000 mg/kg rosemary or green tea extracts for 7 days at 60ºC. *Significance at $P < 0.05$. M0: control sample; R1: 1000 mg/kg rosemary extract; R4: 4000 mg/kg rosemary extract; G1: 1000 mg/kg green tea extract; G4: 4000 mg/kg green tea extract

The rancimat values were not significantly different for all samples pre-treated with rosemary and green tea extracts at all concentrations on day 1. Nevertheless, after 7 days storage at 60ºC, the green tea and rosemary pre-treatments at 4000 mg/kg presented rancimat values of 2.37 ± 0.07 and 2.39 ± 0.09 (h), respectively, while 1.41 ± 0.01, 1.48 ± 0.08 and 1.790 ± 0.09 (h) were detected for the control sample, pre-treatment with rosemary and green tea extracts at 1000 mg/kg. Notably, after 7 days incubation, the rancimat values of the samples supplemented with green tea and rosemary extracts at 4000 mg/kg maintained at 82 and 92% compared to their initial samples. Similar results were reported by Burkow *et al*. [70] that increased antioxidant concentration gave increased induction times for all the antioxidant systems tested, based on rancimat measurements.

Rosemary extract and green tea extract addition significantly increased significantly induction time values [71], especially for high concentrations of 5000 mg/kg [35].

3.2 The comparison of natural green tea and rosemary extracts with synthetic antioxidants

3.2.1 Peroxide value, carotenoids content and rancimat value

The protective effects of natural antioxidants (4000 mg/kg rosemary and green tea extract) in comparison with synthetic antioxidants (150 mg/kg BHT and BHA) for gac oil are demonstrated in Table 1.

Table 1. Peroxide value, carotenoids content and rancimat values of samples treated with BHA, BHT, rosemary and green tea extracts after 7 days storage at 60°C

Sample	Quality index			Protection factor
	Peroxide value (meq/ kg)	Carotenoid content (mg / g)	Rancimat value (h)	
Control	7.50 ± 0.40^{e}	1.20 ± 0.04^{e}	1.41 ± 0.01^{c}	1.00 ± 0.01^{c}
BHA 150 mg/kg	4.36 ± 0.01^{b}	$1.48 \pm 0.02^{a,b}$	2.31 ± 0.01^{a}	1.64 ± 0.01^{a}
BHT 150 mg/kg	4.71 ± 0.06^{c}	$1.43 \pm 0.01^{a,b}$	2.38 ± 0.08^{a}	1.69 ± 0.06^{a}
Rosemary extract 1000 mg/kg	5.90 ± 0.03^{d}	$1.36 \pm 0.01^{c,d}$	1.48 ± 0.08^{c}	1.05 ± 0.06^{c}
Rosemary extract 4000 mg/kg	4.90 ± 0.07^{c}	$1.51 \pm 0.00^{a,b}$	2.39 ± 0.09^{a}	1.70 ± 0.06^{a}
Green tea extract 1000 mg/kg	$4.20 \pm 0.03^{a,b}$	$1.27 \pm 0.01^{d,e}$	1.81 ± 0.09^{b}	1.27 ± 0.06^{b}
Green tea extract 4000 mg/kg	3.97 ± 0.07^{a}	1.55 ± 0.01^{a}	2.37 ± 0.07^{a}	1.68 ± 0.05^{a}

Note: Data are expressed as mean values ± standard deviation of three independent experiments. Similar or shared superscript letters in the same column (e.g., a and a,b) show that there are no significant differences between samples treated with/without antioxidants based on post-hoc test. Different superscript letters (e.g., a and b) show the statistical difference ($p \leq 0.05$) between the tested groups.

The data clearly showed that the protective ability of these natural antioxidants against oxidative reactions of gac oil as measured by PV, carotenoids content and rancimate value were similar to synthetic antioxidants. In particular, supplementation with green tea extract resulted in lower PV than treatments with BHT or BHA. The protection factors of added antioxidants were also calculated (Table 1). As expected from the induction time, there were no significant differences between the protection factors calculated for BHT, BHA, and green tea and rosemary extracts at 4000 mg/kg. According to Nogala-Kalucka *et al*. [46], rosemary extract gave a markedly greater protection factor than α, δ, γ-tocopherol, and BHT at 100 mg/kg for triacylglycerols in rapeseed oil. In other studies, the protection factors were 1.1, 1.3, 1.2 and 1.2 for α, γ-tocopherol, BHT and BHA at 200 mg/kg in peanut oil, respectively, whereas rosemary extract gave values of 1.3-1.4 depending on the percentage of carnosic acid [72]. The differences may be due to the different concentrations of added antioxidants, active components contained in the extracts, and the type of oil used in these studies.

Wanasundara and Shahidi [25] demonstrated that green tea extract at 1000 mg/kg showed the better protection than did α-tocopherol, BHA and BHT, as indicated by lower records of PV. In addition, green tea extract also showed a superiority of protection against oxidation in comparison

to rosemary extract [22]. Rosemary extract also exhibited powerful antioxidant activity, almost equal to that of synthetic antioxidants (BHA and BHT) [40]. However, the opposite result observed for the oil stored at 20 ± 2ºC indicates that the antioxidant effectiveness of rosemary extract was better than that of green tea extract although both antioxidants managed to retard the oxidation process in the initial storage at higher concentration of 2500 mg/kg [35].

In some previous reports, Unten *et al*. [73] demonstrated the suppressive effect of tea polyphenols on the degradation of β carotene in both water-soluble and oily systems. Mohamed [64] also demonstrated that green tea, which is rich in flavanols, had a higher ability to inhibit the decomposition of β carotene than did rosemary extract.

According to Yang *et al*. [74], the induction time value of oil with combined rosemary extract was significantly higher than that with added synthetic antioxidants, which suggested the higher antioxidant effectiveness of rosemary extract against deterioration compared to synthetic antioxidants. Similar results obtained for ghee, both green tea and rosemary extracts at 0.5% concentration gave induction time value higher than BHA [34].

3.2.2 Sensory analysis

The sensory scores of all samples generally decreased after 7 days of incubation at 60ºC (Table 2). At day 1, no significant differences in taste, aroma and overall acceptability found among treatments with BHA and BHT were found. However, rosemary and green tea treatments had an effect on odor in gac oil, which was evaluated by the assessors. The higher concentration of these plant extracts markedly decreased the sensory scores of samples. After 7 days, rosemary treatment (4000 mg/kg) showed the worst results in overall acceptability while lower concentration (1000 mg/kg) resulted in better sensory scores. Green tea (4000 mg/kg) showed the highest overall acceptable by the assessors than BHT, BHA and the other treatment samples.

Table 2. Changes in sensory properties of gac oil after 7 days storage at 60ºC

Sample	Day	Taste	Aroma	Overall acceptability
Control	1	$7.3 \pm 0.5^{b,c}$	7.9 ± 0.3^{a}	$7.7 \pm 0.5^{a,b}$
BHA 150 mg/kg	1	$7.3 \pm 0.7^{b,c}$	6.8 ± 0.4^{b}	$7.3 \pm 0.5^{b,c}$
BHT 150 mg/kg	1	$7.4 \pm 0.5^{b,c}$	6.9 ± 0.3^{b}	$7.1 \pm 0.3^{c,d}$
Rosemary extract 1000 mg/kg	1	8.1 ± 0.9^{a}	7.9 ± 0.9^{a}	7.9 ± 0.6^{a}
Rosemary extract 4000 mg/kg	1	6.6 ± 0.5^{d}	6.8 ± 0.8^{b}	6.8 ± 0.4^{d}
Green tea extract 1000 mg/kg	1	$7.7 \pm 0.8^{a,b}$	7.5 ± 0.5^{a}	$7.7 \pm 0.8^{a,b}$
Green tea extract 4000 mg/kg	1	$7.1 \pm 0.3^{c,d}$	6.8 ± 0.6^{b}	$6.9 \pm 0.6^{c,d}$
Control	7	6.7 ± 0.5^{c}	$6.9 \pm 0.3^{a,b}$	$7.1 \pm 0.6^{b,c}$
BHA 150 mg/kg	7	6.7 ± 0.7^{c}	6.6 ± 0.5^{b}	$6.7 \pm 0.7^{c,d}$
BHT 150 mg/kg	7	6.7 ± 0.9^{c}	6.6 ± 0.7^{b}	$6.7 \pm 0.5^{c,d}$
Rosemary extract 1000 mg/kg	7	7.9 ± 0.9^{a}	7.3 ± 0.5^{a}	$7.3 \pm 0.7^{a,b}$
Rosemary extract 4000 mg/kg	7	6.3 ± 0.7^{c}	$6.8 \pm 0.8^{a,b}$	6.4 ± 0.7^{d}
Green tea extract 1000 mg/kg	7	$6.9 \pm 0.6^{b,c}$	6.5 ± 0.7^{b}	$6.6 \pm 0.7^{c,d}$
Green tea extract 4000 mg/kg	7	$7.5 \pm 0.8^{a,b}$	7.3 ± 0.5^{a}	7.7 ± 0.7^{a}

Note: Data are expressed as mean values ± standard deviation of three independent experiments. Similar or shared superscript letters in the same column (e.g., a and a,b) show that there are no significant differences treated with/without antioxidants based on post-hoc test. Different superscript letters (e.g., a and b) show the statistical difference ($p \leq 0.05$) between the tested groups.

According to Pourashouri *et al*. [75], a low percentage of rosemary extract addition had no effects on sensory evaluation but treatments with higher concentrations gave higher score for off-flavor. Moreover, these volatile including aldehydes, hydrocarbons and sulphuric compounds usually emerge during storage time due to lipid peroxidation [76]. Based on Table 1, green tea supplementation at 4000 mg/kg, which resulted in the lowest PV, contributed to it having highest overall acceptability scores when compared to the other treatments after the storage. Although some reports suggested that there was a slight increase in rancid odor when rosemary and green tea extracts were used [75, 77]. Overall, these plant extracts prevent alterations caused by oxidation which may affect the sensory scores of gac oil.

4. Conclusions

For the first time, the effects of two types of natural antioxidants, rosemary extract and green tea extract, on gac oil were carried on. Supplementation with rosemary and green tea extracts at 4000 mg/g significantly improved the quality of the gac oil through a decrease in peroxide value and maintenance of carotenoid during storage. A comparison between natural and synthetic antioxidants (BHA and BHT) was also performed to evaluate suitable antioxidants for gac oil. Both rosemary and green tea extracts showed impressive results for gac oil protection against oxidation. Although rosemary and green tea somewhat have an off-odor effect on gac oil, these results propose the possibility of replacing BHA and BHT by green tea and rosemary extract. Green tea seems to be a promising candidate in gac oil preservation.

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