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Research Article:

Evaluation of the effect of passion fruit (*Passiflora edulis*) leaf extract on the oxidative stability of palm oil during heating

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Abstract

The growing need for natural alternatives to synthetic antioxidants in edible oils has driven extensive research into plant-derived extracts for enhancing oil stability during thermal processing. This study was carried out to evaluate the effect of continuous heating on the quality of palm oil and to assess its oxidative stability during heating by adding passion fruit leaf extract as an antioxidant. Acetone was used as the solvent to extract antioxidant components from fresh leaves with a sample to solvent ratio of 1:10 (w/v). Negative control (oil samples without any added antioxidants), positive control (oil samples added with 200 ppm of Butylated Hydroxytoluene (BHT) and test samples (oil samples added with 1000 ppm of leaf extract) were prepared. All oil samples were evaluated for stability under continuous heating at 170±5 °C. Continuous heating was carried out for up to 24 h (30 min heating followed by 30 min cooling), that is, 24 heating cycles. Sampling was done at 2 h intervals (after every 2 heating cycles). The levels of oxidation of the samples were determined by evaluation of peroxide value, *p*-anisidine value, TOTOX value, free fatty acid content, total polar compounds, fatty acid composition and conjugated diene (CD) and conjugated triene (CT) values. All parameters measured were increased in all three samples; however, free fatty acid content, peroxide value, *p*-anisidine value and CD and CT values were significantly less in the test samples than in the positive control and negative control. The average rate of total polar compound formation, expressed as the percentage increase per heating cycle, was significantly lower in extract-treated (1.29% per cycle) and positive control (1.27% per cycle) samples than in the negative control (1.68% per cycle). These results indicate that the sample supplemented with passion fruit leaf extract exhibited higher thermal stability than both controls, and that fresh leaf extract (1000 ppm) more effectively controls the thermal oxidation of palm oil than BHT during continuous heating.

Keywords: Heating, Leaf extract, Palm oil, Passion fruit, Thermal stability.

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1. Introduction

Lipid oxidation is one of the major causes of deterioration in edible oils. Fatty acid composition and the presence of minor compounds are the primary factors that affect lipid oxidation (Madhujith & Sivakanthan, 2018). Lipid oxidation products may be harmful to human health, as they may have mutagenic, carcinogenic, and cytotoxic properties (Domínguez et al*.,* 2019).

High-temperature processing (eg, frying) is the most common culinary practice involving edible oils. Oils used in frying are prone to oxidation, hydrolysis, and polymerization, driven by factors such as temperature, oxygen, and moisture, leading to physical and chemical changes that result in undesirable changes in functional, sensory, and nutritive values of the oil as well as fried foods (Silva et al., 2024). During high-temperature processing, the concentration of free radicals increases. Therefore, the oil used for thermal processing at high temperatures should be able to withstand high temperatures. It can be indicated as the stability of the oil against oxidation and other changes. Given the stability of edible oils, the degree of saturation is a major concern when choosing a suitable oil for different processing applications. Polyunsaturated fatty acids are more susceptible to oxidation than monounsaturated fatty acids and saturated fatty acids (Tarmizi et al., 2016). However, polyunsaturated fatty acids provide several health benefits. Therefore, it is necessary to protect the polyunsaturated fatty acids from destruction during processing. To inhibit oxidation in edible oils, antioxidants can be used. Among synthetic antioxidants used in food lipids, butylated hydroxyltoluene (BHT) and butylated hydroxylanisole (BHA) are most widely used. However, recent reports suggest that these synthetic antioxidants are associated with adverse health effects in humans (Ren et al., 2025). As a result, most research focuses on using natural antioxidants to improve the oxidative stability of oils. It has been demonstrated that most natural antioxidants exhibit greater antioxidant activity and thermal stability than synthetic antioxidants (Ali et al., 2026; Petcu et al., 2023; Pop & Dippong, 2024; Zhao et al., 2025). In this backdrop, the present study was designed to evaluate a plant-based natural antioxidant in widely used edible oils in Sri Lanka. Therefore, palm oil was selected as the edible oil, and passion fruit leaves were selected as the source of natural antioxidants.

Palm oil is one of the most widely used edible oils in Sri Lanka, especially for deep frying in commercial and domestic settings due to its favourable frying performance and cost-effectiveness. Despite its widespread use, repeated heating of palm oil, as commonly practised in eateries and households, accelerates oxidation and the formation of potentially harmful compounds. The choice of palm oil in this study reflects its dominant role in local frying practices and the relevance of improving its oxidative stability under high-temperature, real-world conditions. For experimental authenticity, the palm oil sample was sourced from an establishment that routinely uses it for frying, ensuring that the oil reflects typical usage scenarios.

Passion fruit (*Passiflora edulis*), belonging to the family Passifloraceae, is widely grown in Sri Lanka and has been recognized for its abundance of bioactive compounds such as polyphenols, alkaloids, and glycosides (da Silva et al*.,* 2013). Several studies have highlighted the antioxidant potential of passion fruit leaves, demonstrating their ability to scavenge free radicals and inhibit lipid peroxidation (da Silva et al., 2013; Ferreres et al., 2007; Ramaiya et al., 2014). However, limited research has assessed their effectiveness as a natural antioxidant in edible oils under high-temperature conditions, particularly in palm oil. By investigating passion fruit leaf extract, this study seeks to expand the understanding of its practical application as a cost-effective, natural antioxidant to enhance the oxidative stability of palm oil during frying.

2. Materials and Methods

2.1 Materials

Mature passion fruit leaves were collected at the Kilinochchi Premises of the University of Jaffna. Only fully expanded, mature leaves were selected, avoiding young or senescent leaves. The collection was performed in the early morning to minimize degradation of phytochemicals, and leaves were immediately cleaned with distilled water after harvesting. Palm oil used in the study was refined and purchased from a shop in Gampaha, Sri Lanka, where it is routinely used for food applications (frying). All chemicals used in this study were of analytical grade.

2.2. Preparation and analysis of leaf extracts

Two types of leaf extracts were prepared: one from dried leaves and one from fresh leaves to identify the extract with the highest antioxidant potential for subsequent incorporation into the oil during heating experiments. Mature passion fruit leaves were collected, cleaned and cut into small pieces (approximately 1 cm × 1 cm) and kept in the oven for 24 h at 40 °C. The dried passion fruit leaves were ground using a domestic grinder to get fine powder, which was stored in ziplock bags at 4 °C until used for the antioxidant extraction. Fresh passion fruit leaves were cleaned and cut into small pieces, and used for fresh sample preparation. Cut fresh passion fruit leaves (5 g) were taken and macerated using a motor and pestle. Then, macerated leaves were transferred into the 250 mL clean conical flask, and 100 mL of acetone was added as the solvent for extraction. For the dry sample, 5 g of dry powder was added to the 250 mL clean conical flask and 100 mL of acetone was added. The extraction process was performed for 2 h in one test and 4 h in another in a shaker at room temperature (200 rpm). The extract was filtered through Whatman No. 1 filter paper. The re-extraction process was also performed on the residue under the same conditions for 30 min, and the filtrates were pooled. The filtrate was transferred into the round-bottom flask, and the solvent was evaporated in a rotary evaporator (EYELA OSB-2100-CE, Japan) at 56 °C. The dry extracts were weighed and stored at -20 °C for subsequent analysis of antioxidant potential and use in heating experiments within two weeks.

2.3 Analysis of extract

2.3.1 Determination of total phenolic content by Folin-Ciocalteau assay

Total phenolic content was determined using Folin-Ciocalteau reagents (Singleton and Rossi, 1985). Gallic acid standard solution (0.25 mg/mL) was prepared by dissolving 25 mg of gallic acid in 100 mL of distilled water. The solution was diluted to prepare solutions of different concentrations. Three hundred microliters (350 µL) of extract were mixed with 1.8 mL of Folin-Ciocalteau reagent and 1.2 mL of sodium bicarbonate (7.5%). An excess of 9.65 mL of distilled water was added and vortexed. The absorbance was measured at 765 nm after standing for 30 min at room temperature. Gallic acid equivalent was calculated using a calibration curve, and the results were expressed as mg gallic acid equivalent (GAE)/g Dry weight (DW).

2.3.2 Determination of total antioxidant capacity by Phosphomolybdenum assay

The antioxidant capacity of the sample was evaluated by using the green phosphomolybdenum complex formation method (Prieto et al., 1999). Sulphuric acid (5 mL), ammonium molybdate (490 mg), and sodium phosphate (360 mg) were used to prepare the reagent solution, and the final volume was adjusted to 100 mL with distilled water. An ascorbic acid standard solution was prepared by adding 25 mg of ascorbic acid and dissolving it in 100 mL of distilled water. Three hundred and fifty microliters of extract were mixed with 1 mL of reagent solution. It was kept in a water bath (Yamato BT200, Japan) at 95 °C for 90 min. The absorbance was read at 695 nm using a UV-Visible spectrophotometer (Thermo Scientific Evolution 200, USA). The phosphomolybdenum reduction potential of extracts was determined (mg ascorbic acid/g) using a calibration curve.

2.3.3 Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The radical scavenging activity was determined by the DPPH assay (Chang et al., 2001) with slight modifications, with ascorbic acid as reference. DPPH solution was prepared by dissolving 6 mg of DPPH in 100 mL of methanol. Different volumes of extract and ascorbic acid standard were made up to 350 µL with methanol. Then, 10.65 mL of excess methanol and 5 mL of DPPH solution were added. The sample was kept in a dark room for 30 min. After 30 min, the absorbance was read at 517 nm using a UV-Visible spectrophotometer (Thermo Scientific Evolution 200, USA). Methanol (11 mL) and DPPH (5 mL) were taken as a control. The percentage of radical scavenging activity of the extract was determined using the following formula;

$$\%RSA = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Where RSA is the Radical Scavenging Activity, Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of extract (DPPH radical + extract or ascorbic acid standard).

2.3.4 Experiments on heating the oil

A heating experiment was conducted on palm oil to determine the effect of adding passion fruit leaf extract. The passion fruit leaf extract, selected according to the results of antioxidant analyses of extracts obtained from both dried leaf powder and fresh leaves, was incorporated into the palm oil at a concentration of 1000 ppm. BHT was also added at its legal limit of 200 ppm (Codex Alimentarius, 2025) as a positive control. After adding the extract/BHT, the oil samples were stirred using a magnetic stirrer bar. The oil without any added antioxidants was used as a negative control. The samples (test, positive control, and negative control) were heated separately at a constant temperature (170±5 °C) in a stainless-steel pan (approximately 3 L capacity). The temperature was maintained at 170±5 °C throughout the heating process and monitored with a thermocouple. The heating was performed for 30 min, followed by 30 min of cooling (one heating cycle). In the same manner, the heating process was carried out to finish 24 heating cycles. The samples were drawn after two frying cycles (2 h intervals), and a total of 12 samples were collected from each experiment. The oil samples were kept at -20 °C for further analysis within two weeks.

2.3.5 Analysis of oil

2.3.5.1 Determination of free fatty acids content

The AOCS official method Ca 5a-40 (AOCS, 2009a) was followed to determine the acid value. An oil sample (3-5 g) was weighed into the dry, clean flask, and 25 mL of ethanol was added. It was stoppered and shaken well to dissolve the oil completely. It was kept in a boiling water bath (Yamato BT200, Japan) for 30 min and cooled. Titration was done against 0.1 N Sodium hydroxide solution using phenolphthalein as an indicator until the pink colour persisted for 30 s. A blank test was also carried out.

2.3.5.2 Determination of peroxide value

The Peroxide value of the sample was measured using the spectrophotometric method (Hornero-Méndez et al., 2001). Fe (II) stock solution was prepared using 0.2 g of $BaCl_2.H_2O$ and 0.25 g of $FeSO_4 \cdot 7H_2O$, each dissolved in 25 mL of deionized water. Concentrated HCl (1 mL) was added to the resulting solution, and the mixture was filtered and stored. The sample (0.01-0.05 g) was added to a 10 mL screw-capped tube and dissolved in 2 mL of chloroform/acetic acid solution (2:3 v/v) with the addition of 200 µL of Fe(III) solution. It was mixed for 15 s using a vortex mixer and left in a dark room for 10 min. Deionized water (2 mL) was added, and then 4 mL of diethyl ether was added. The organic phase was discarded, and the remaining ether in the aqueous phase was removed. One milliliter of the aqueous phase was transferred to another tube and mixed with 50 µL of saturated ammonium thiocyanate solution. Then, 2 mL of deionized water was added. A control experiment was carried

out. After 10 min, absorbance at 470 nm and 670 nm was measured against a blank (deionized water) using a UV-visible spectrophotometer (Thermo Scientific Evolution 200, USA). The peroxide value of the samples in meq/kg sample was calculated as follows;

$$Peroxide\ value = \frac{As470nm - Ab470nm}{55.84 \times 2 \times m \times W} - \frac{As670nm - Ab670nm}{55.84 \times 2 \times m \times W}$$

Where As 470 nm is the absorbance of the sample at 470 nm; Ab 470 nm is the absorbance of the control at 470 nm; As 670 nm is the absorbance of the sample at 670 nm; Ab 670 nm is the absorbance of the control at 670 nm; m is the slope of the Fe (III) calibration curve; 55.84 is the atomic weight of Fe, 2 is the factor to convert milliequivalents (meq) of Fe to milliequivalents of peroxide and W is the sample weight (g).

2.3.5.3 Determination of *p*-anisidine value

The *p*-anisidine value was determined according to AOCS official method Cd 18-90 (AOCS, 2009b). About 1±0.05 g of sample was measured into a 25 mL volumetric flask and dissolved. The sample was diluted using iso-octane. The absorbance was measured at 350 nm using a UV-Visible spectrophotometer (Thermo Scientific Evolution 200, USA). Iso-octane was used as the blank solution. Five milliliters of the sample solution and 5 mL of the solvent were added to a test tube, and 1 mL of *p*-anisidine reagent was added to the mixture and mixed. The absorbance was measured at 350 nm. The *p*-anisidine value was calculated using the following formula.

$$p - anisidine\ value = \frac{25 \times (1.2As - Ab)}{m}$$

Where As is the absorbance of the fat solution after reaction with the *p*-anisidine reagent, Ab is the absorbance of the fat solution, and m is the mass of the sample (g).

2.3.5.4 Determination of Total Oxidation values (TOTOX)

TOTOX values of oil samples were calculated by using the following formula

$$TOTOX\ value = (2 \times peroxide\ value) + p - anisidine\ value$$

2.3.5.5 Determination of Conjugated Dienes (CDs) and Conjugated Trienes (CTs)

CD and CT values were determined using the IUPAC analytical method (IUPAC, 1987), with modifications. Oil sample (1±0.005 g) was mixed with 25 mL of iso-octane in a vortex mixer for 15 s. The absorbance was measured at 235 nm for CD and 270 nm for CT using a UV-Visible spectrophotometer (Thermo Scientific Evolution 200, USA). The calculation was done according to the following formula,

$$\%E1 = \frac{A\lambda}{C \times 1}$$

Where Aλ is the absorbance measured at either 235 nm or 270 nm, C is the concentration of oil solution in (g/100 mL), and l is the length of the cuvette in cm.

2.3.5.6 Determination of nonpolar and polar compounds

Column chromatography was used to separate the polar and nonpolar compounds in the frying oils according to the IUPAC method (IUPAC, 1992) using silica gel (70-230 mesh) as the stationary phase by gradient elution with a mixed solution of light petroleum ether and diethyl ether, 90/10 (v/v) (solvent 1) and diethyl ether (solvent 2). Briefly, the oil sample (1±0.1 g) was dissolved in solvent 1 in 10 mL volumetric flask. The sample was introduced into the column (20 mm diameter and 300 mm length), and the nonpolar fraction was eluted using elution solvent 1 (60 mL), followed by the polar fraction using elution solvent 2 (250 mL). The flow rate was maintained at 1.5 mL/min. The mass fraction of polar compounds was calculated as a percentage.

2.3.5.7 Determination of fatty acid profile using Gas-Liquid Chromato- graphy (GLC)

The fatty acid profile of the samples was analyzed using Gas-Liquid Chromato- graphy. FAMEs were prepared according to WHO

(2020), and FAMEs were analyzed by injecting 1 µL into GLC (Agilent Technologies 7890B GC system), equipped with a Flame Ionization Detector (FID) and a fused silica capillary column (100 m length, 0.25 mm diameter and 0.20 µm film) using nitrogen as carrier gas at a flow rate of 20 mL/min. Split ratio was 30:1. Injector and detector temperatures were maintained at 260 °C. The initial column oven temperature was maintained at 140 °C for 5 min, then increased to 240 °C at 4 °C/min and held at 240 °C for 10 min. Fatty acids were identified using authentic standards (Supelco 37-component FAME mixture and *trans* isomers of linoleic acid).

2.4 Statistical analysis

Experiments were carried out in triplicate. Analysis of Variance (ANOVA) was calculated using the Statistical Analysis System (SAS) using a statistical model as a Two Factor Completely Randomized Design. Duncan's Multiple Range Test (DMRT) was used to compare the treatment means ($p<0.05$). The correlation between the parameters was determined using Spearman's correlation coefficient in Microsoft Excel 2007. The data were presented as the mean and standard deviation using Microsoft Excel 2007.

3. Results and Discussion

3.1 Antioxidant properties of passion fruit leaf extract

Determination of the antioxidant properties of the leaf extract was carried out to select the best source (fresh leaf extract or dry leaf extract) to incorporate into the oil sample used in this study. Acetone was used as the solvent for the extraction process because, in this study, the aim was to incorporate leaf extract into palm oil, and acetone can extract the antioxidant compounds soluble in the oil from the leaf. The total phenolic content, total antioxidant capacity, and DPPH radical scavenging activity of fresh and dry leaf extracts with duration of extraction are shown in Table 1.Total antioxidant activity of the extracts is expressed as an IC_{50} value, which is inversely proportional to antioxidant activity. That is, the higher the IC_{50} value, the smaller the antioxidant activity. According to the results, the fresh leaf extract obtained after 4 h of extraction exhibited the highest antioxidant activity. Therefore, the extract obtained from fresh leaves after 4 h of extraction was used for further experiments.

3.2 Effect of the addition of passion fruit leaf extract on the oxidative stability of palm oil during continuous heating

The effect of the addition of fresh passion fruit leaf extract on the oxidative stability of the palm oil was determined. The leaf extract was added to the oil at 1000 ppm. Since there is no legal limit for the use of natural antioxidants, 1000 ppm of the plant extract was used and compared with BHT at 200 ppm.

The initial quality of the oil was determined using free fatty acid content and peroxide value. According to the Codex standard, the maximum free fatty acid value of palm oil should be 5% (Codex Alimentarius, 2016). The sample showed a free fatty acid content of nearly 44.76%.

Table 1: Total phenolic content, total antioxidant capacity and DPPH radical scavenging activity of fresh and dry leaf extracts

Duration of extraction (h)	Total phenolic content (mg GAE/g DW)		Total antioxidant capacity (mg AAE/g DW)		Total antioxidant activity (IC_{50} value mg/mL)	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
2	41.21 ± 1.83^{Ab}	44.39 ± 1.17^{Aa}	30.60 ± 1.55^{Aa}	23.67 ± 0.58^{Aa}	0.25 ± 0.6^{Aa}	0.23 ± 0.1^{Aa}
4	47.32 ± 0.96^{Ba}	44.49 ± 1.56^{Ab}	48.35 ± 0.21^{Aa}	30.90 ± 1.37^{Bb}	0.17 ± 0.05^{Ba}	0.12 ± 0.04^{Ba}

Values are means ± standard deviation for duplicate analyses. Means in each row followed by different superscript letters (a-e) are significantly different ($p<0.05$). Means in each column followed by different superscript letters (A-E) are significantly different ($p<0.05$).

It indicates that the palm oil sample used in this study has undergone extensive hydrolysis and is therefore of very poor quality. The peroxide value of palm oil (0.49 meq/kg) was lower than the Codex standard value (10 meq/kg of oil). This indicates that the oil oxidation level was lower, or that the peroxides produced during oxidation, as primary oxidation products, may have decomposed into secondary oxidation products. The *p*-anisidine value of the oil was 52.42, indicating a significant quantity of secondary oxidation products. Trivedi et al. (2017) reported an initial *p*-anisidine value of 7.05 for palm olein oil, which is much lower than the value reported in the present study.

In this study, palm oil was sourced directly from a local shop, rather than laboratory-grade or specially refined oil, to closely replicate real practices in Sri Lanka. Using oil commonly employed in commercial food preparation enhances the practical applicability of the findings of this study. This approach ensures that the results are more directly relevant to industry contexts.

3.2.1 Free fatty acid content

The formation of free fatty acids occurs during thermal processing due to the hydrolysis of lipids at high temperatures. Lipid oxidation also produces free fatty acids (Asadia and Farahmandfar, 2020). The level of free fatty acid (as % of palmitic acid) for the oil samples is shown in Figure 1. The free fatty acid content was increased significantly for all three samples. This indicates that oxidation and hydrolysis levels gradually increased with the number of heating cycles in palm oil. Similar results have been reported in other studies. For example, Trivedi et al. (2017) reported that the free fatty acid content in palm oil samples increased with increased frying. The frying process adds moisture from the food into the oil, which accelerates the formation of free fatty acids. In the present study, the oil is only heated; however, high temperatures can also increase the rates of hydrolysis and oxidation of the oil (Choe and Min, 2007) and increase the free fatty acid content.

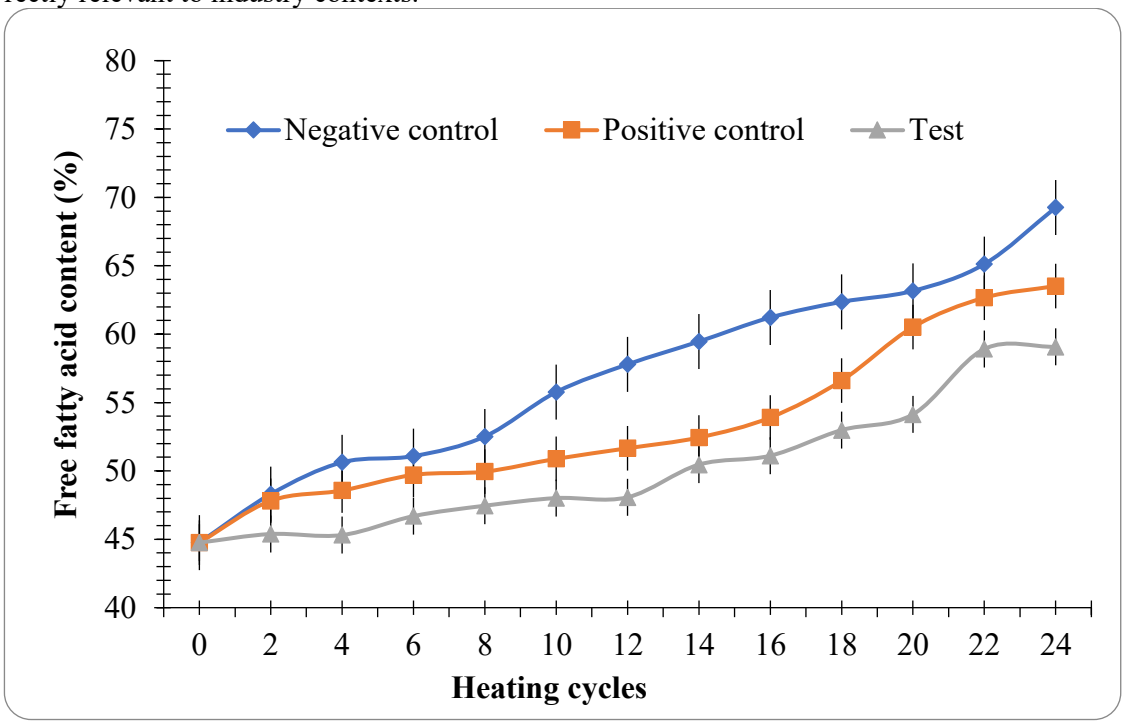

Figure 1: Changes in free fatty acid content in oil samples during heating

Irrespective of the treatment applied, an increase in the free fatty acid content was observed in all samples during heating. This trend is likely attributable to the initial quality of the oil, which has already undergone some degree of degradation before experimentation. The subsequent thermal treatment applied during the experiments could have further accelerated hydrolysis and oxidative breakdown of triglycerides, resulting in increased free fatty acid formation in all samples. However, the negative control showed a significantly higher free fatty acid content than the positive control and the test, whereas the sample with the leaf extract (test) showed lower free fatty acid content than the controls. The results indicate that the addition of passion fruit leaf extract at 1000 ppm is more effective in decreasing the formation of free fatty acids during continuous heating than BHT.

3.2.2 Peroxide value

Peroxide value is an indicator of the degree of primary oxidation of oil and measures the number of primary intermediates formed during lipid oxidation, such as peroxides (Kitts et al*.,* 2019). Peroxide value indicates the formation of peroxides during continuous heating. Figure 2 depicts the changes in peroxide value of the samples during heating. The peroxide value increased significantly with heating cycles across all samples, indicating that oxidation increased with heating time. The negative control showed the highest level of peroxides, significantly higher than those of the positive control and the test samples. The test sample exhibited the lowest peroxide values throughout the experiment, indicating the effectiveness of the extract in inhibiting palm oil oxidation. Up to the 12th heating cycle, the peroxide values of the test and positive controls increased significantly but slowly; however, after the 12th heating, the rate of increase in the peroxide value of both was high. In the negative control, a steep increase in the peroxide value was observed from the 6th heating cycle. This observation aligns with the results reported in previous studies. For example, Zhao et al. (2025) reported that the peroxide value of camellia seed oil without antioxidant addition subjected to 180 ◦C heating exhibited a time-dependent increase from an initial value of 0.4 meq O2/kg to 7.67 meq O2/kg after 3 h thermal treatment. The steep increase in the peroxide value of the negative control from the 6^{th} heating cycle aligns with results reported by Zhao et al. (2025), who found that camellia seed oils exceeded stable limits quickly under heating at 180 °C. In contrast, the addition of antioxidants such as BHA, tert-butylhydroquinone (TBHQ), epigallocatechin gallate and α-tocopherol effectively delayed this process by acting as free radical scavengers or hydrogen donors that disrupt the propagation phase of oxidation. The negative control exhibited the highest levels of peroxides because its native unsaturated constituents are highly susceptible to this rapid oxidative degradation. The effectiveness of the test sample in maintaining the lowest peroxide values throughout the experiment is supported by findings that natural phenolic compounds, such as those found in pomegranate flower or burdock extracts, can significantly mitigate the formation of primary oxidation products (Ali et al*.,* 2026; Pop and Dippong, 2024). The observation that the test and positive controls (typically synthetic antioxidants like BHT or TBHQ) increased slowly until a specific point (the 12^{th} cycle) indicated a prolonged induction period during which the antioxidants are actively neutralizing radicals. However, the subsequent high rate of increase suggested that the antioxidants may eventually be depleted or thermally decomposed, causing the oil to lose its oxidative stability.

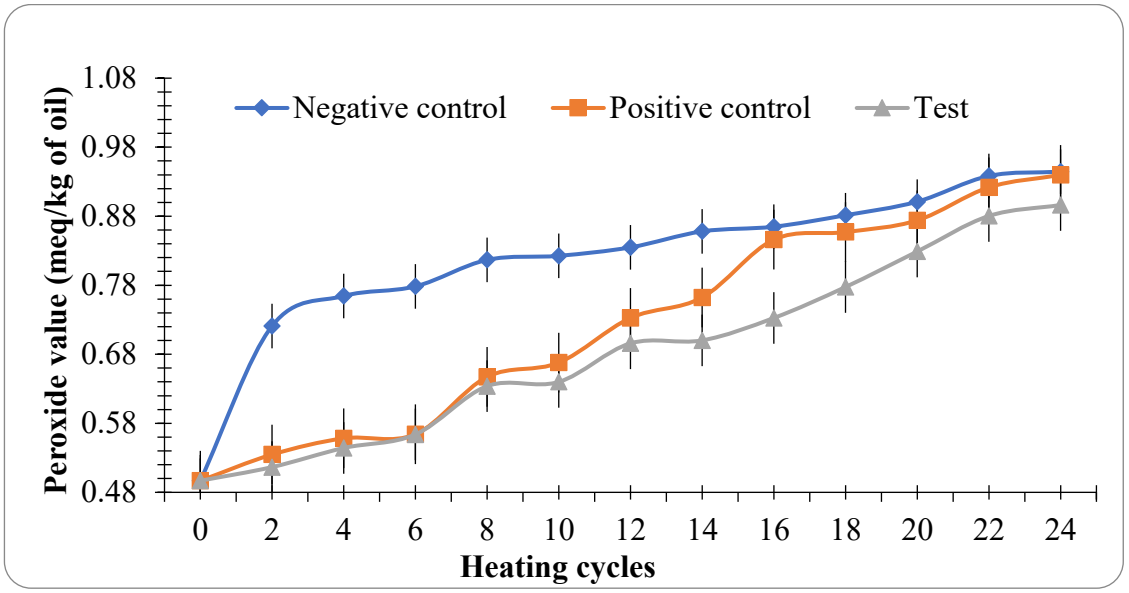

Figure 2: Changes in peroxide value in oil samples during heating

3.2.3 CD and CT values

Conjugated dienes and trienes are oxidation products and indicators of oxidative stability in frying oils (Aydeniz and Yilmaz, 2016). Conjugated diene content is affected by fatty acid composition of the oils, and conjugated diene and triene levels of oils increase with increasing level of unsaturated fatty acids (Abdulkarim et al., 2007). In the present study, CD and CT values (Figures 3 and 4) of all samples increased with increasing heating cycles. The negative control showed significantly higher CD and CT values than the positive control and the test. The oil added with the extract exhibited significantly lower formation of CD and CT than controls. The progressive increase in CD and CT values observed with heating cycles reflects the progress of lipid oxidation, moving from the formation of unstable hydroperoxides to secondary carbonyl degradation products. This thermal degradation has been reported. For instance, Resende et al. (2019) observed that CD and CT values in avocado oil increased significantly at higher temperatures, with rate constants for secondary products increasing 5,300-fold when heated to 100 °C. The efficacy of natural plant extracts in mitigating this formation is supported by Iqbal and Bhanger (2007), who demonstrated that 1000 ppm of garlic extract was the most effective concentration for stabilizing sunflower oil, significantly reducing both CD and CT levels and performing better than synthetic antioxidants such as BHA and BHT.

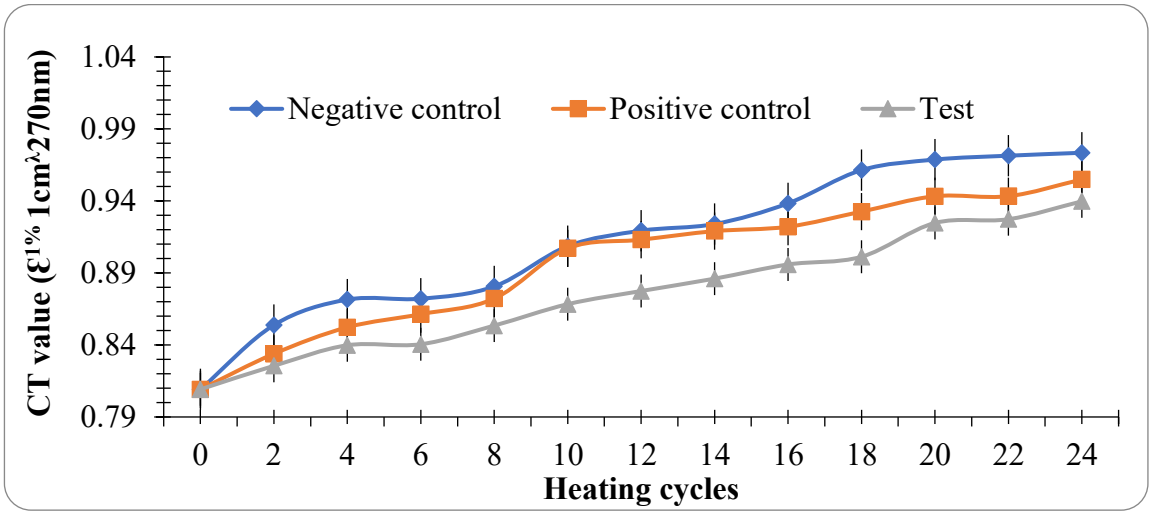

Figure 3: Changes in CT value in oil samples during heating

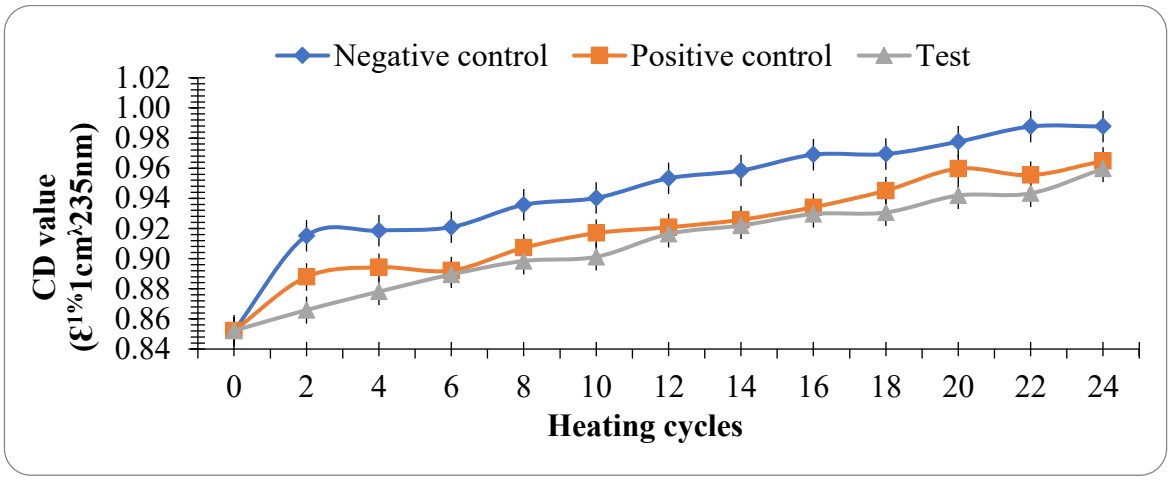

Figure 4: Changes in CD value in oil samples during heating

3.2.4 Total polar compounds

Polar compounds are formed in edible oils from thermal hydrolytic and oxidative alteration. Determination of total polar compounds in frying oils and fats provides reliable information on the extent of deterioration. The level of polar compounds is a good indicator of the quality of frying oils and fats and provides information on the total amount of newly formed compounds that are more polar than triacylglycerols (Abdulkarim et al., 2007). Figure 5 clearly shows that polar compound formation increased with increasing heating temperature across all samples. Asadi and Farahmandfar (2020) also reported similar results: *Teucrium polium* extract demonstrated the ability to reduce the formation of polar compounds during frying, and 25% of polar compounds were formed after 30 h of frying. The acceptable maximum limit of polar compound formation during frying is 24-27% (Yu et al., 2018). This level is considered the rejection level for the frying oils. According to the results of the present study, the negative control sample showed higher polar compound formation than the positive control and test samples. The negative control reached that maximum after 4 heating cycles, whereas the positive

sample reached it after 6 heating cycles. The test sample reached the maximum acceptable level after 10 heating cycles. The delay in the formation of total polar compounds in the test sample indicates that the extracts are highly effective at retarding the formation of polar compounds during the thermal processing of oils. The result that the negative control exceeded safety thresholds more rapidly (after only four cycles) than the extract-treated sample (10 cycles) is consistent with findings by Ali et al. (2026), who reported that sunflower oil without added antioxidants reached total polar compounds of 34% after five days of heating at 180°C, whereas pomegranate flower extract limited this formation to 15%. This protective effect is attributable to the phenolic antioxidants present in the extracts, which function as effective hydrogen donors and free radical scavengers.

3.2.5 TOTOX value

The TOTOX index is a good indicator of the total deterioration of fats and oils. TOTOX value is the combination of both primary and secondary oxidation, and it reflects the accumulation of both hydroperoxide and aldehyde in heated oils (Kitts et al., 2019). According to the results shown in Figure 6, the TOTOX value increased with the heating cycle, and the negative control showed a significantly higher value than the positive and test samples. The total deterioration level of the oil increased at a higher rate in the negative control than in the other two samples. The test showed a lower TOTOX value than controls, and the addition of leaf extract controlled the deterioration of oil during heating.

3.2.6 *p*-Anisidine value

The *p*-anisidine value indicates secondary products formed during lipid oxidation. The *p*-anisidine value method measures the extent of secondary oxidation due to hydroperoxide decomposition (Kitts et al., 2019). *p*-Anisidine is a common reagent used to measure secondary lipid oxidation by quantifying carbonyls, especially unsaturated aldehydes. Aldehydes are secondary oxidation products produced during the oxidation of lipids. According to the results (Figure 7), the *p*-anisidine value increased with the number of heating cycles for all samples. The negative control showed a significantly greater difference than the positive control and the test. The positive control and the test showed lower changes than the negative control. Ali et al. (2026) also reported similar results when pomegranate flower extract was added to sunflower oil. This could be due to the antioxidants in the extract acting as potent hydrogen donors to quench radicals formed during oxidation, thereby preventing the degradation of primary products into the aldehydes measured by the *p*-anisidine assay. The results for the above-discussed parameters clearly reveal that the passion fruit leaf extract at 1000 ppm is more effective than BHT at 200 ppm. This observation is consistent with many other studies that have examined the effectiveness of various plant extracts as natural antioxidants in edible oils during thermal processing. Some recent examples include a study on the use of leaf extracts from *Cressa cretica* against oxidation of soybean oil during thermal processing, which indicated that the leaf extract was more effective at 200-1000 ppm than BHT at 200 ppm (Afshari and Sayyed-Alang, 2016). Another study by Asnaashari et al. (2015) reported the highest efficiency of 1000 ppm of the raspberry (*Rubus fruticosus*) leaves extract compared to BHT and BHA during the frying of sunflower oil. These results indicate that the plant extracts possess higher thermal stability than synthetic antioxidants and, thus, are effective replacements for them.

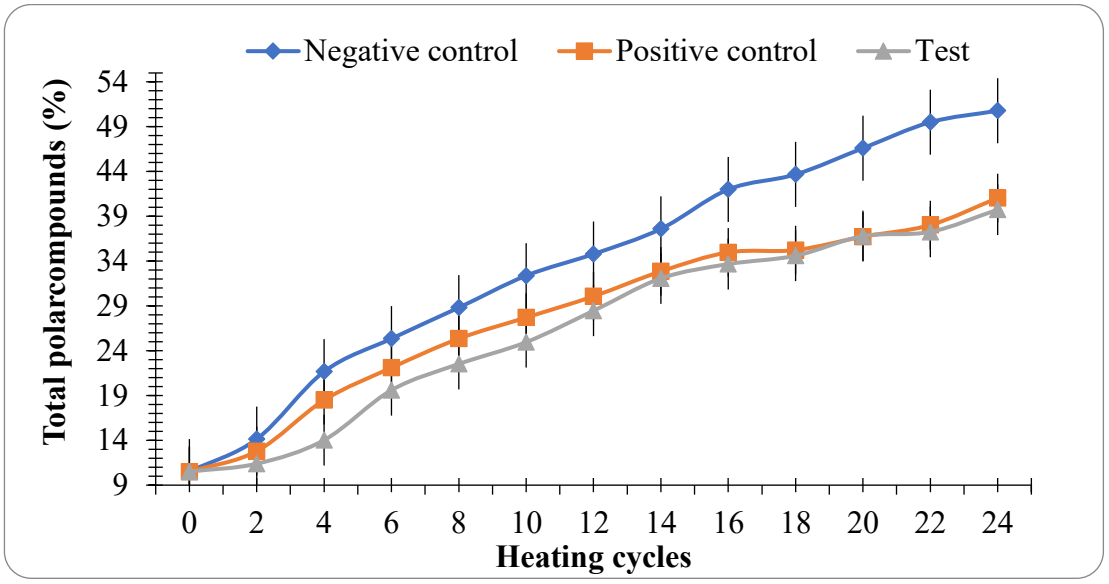

Figure 5: Changes in total polar compounds in oil samples during heating

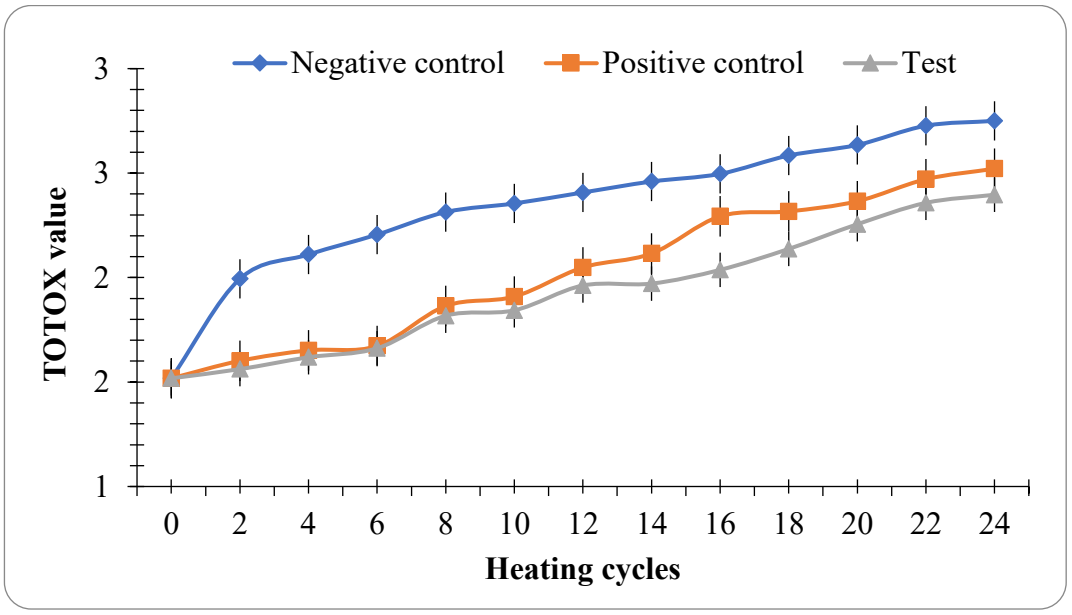

Figure 6: Changes in TOTOX value in oil samples during heating

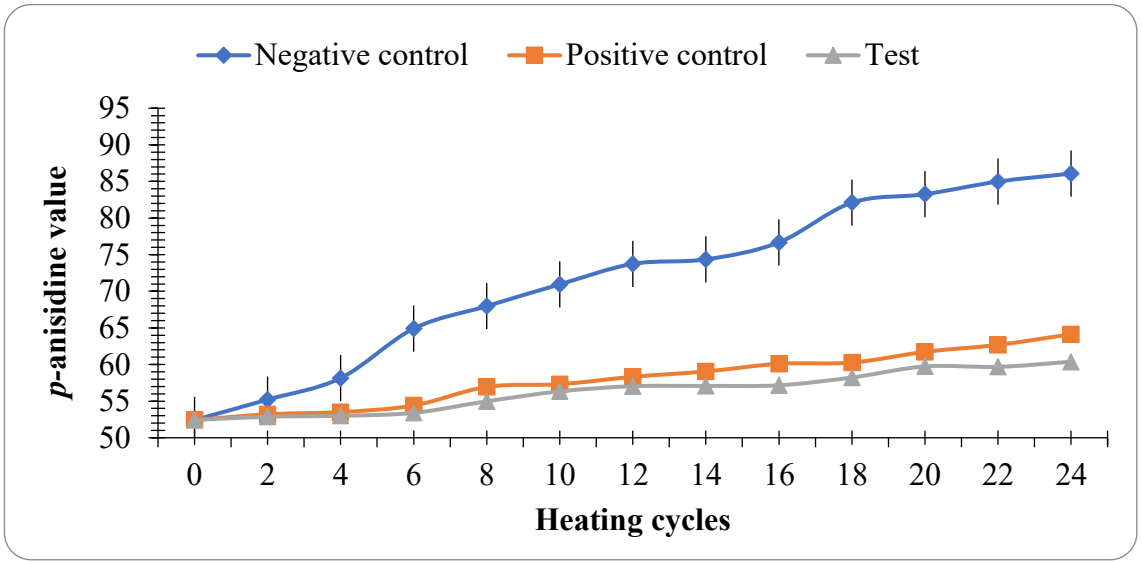

Figure 7: Changes in *p*-anisidine value in oil samples during heating

3.2.7 Correlation analysis of the chemical parameters

The correlation coefficient is a measure of the linear correlation between two variables. Tables 2, 3 and 4 show the correlation coefficients (Spearman's correlation) between the measured chemical parameters in all three samples. The correlation analysis reveals strong positive correlations among the parameters measured during oil heating.

3.2.8 Fatty acid composition

During the thermal processing, changes in fatty acid composition occurred in all three samples. As shown in Tables 5, 6, and 7, the major fatty acids in palm oil are palmitic acid and oleic acid. Major changes occurred in linoleic acid (C118:2) and linolenic acid (C18:3). Both fatty acid contents decreased significantly in all three samples; however, the change was less significant in the test (extract-added oil sample), followed by the positive control. This indicates that the leaf extract had the potential to inhibit the oxidation of these fatty acids to a greater extent than BHT. *Trans* fat formation can also be seen during heating cycles in all samples. Before starting the heating, trans fatty acids have also been detected in small quantities. Formation of *trans* fatty acids was also less significant in the test sample compared to controls. Previous studies have also reported that natural antioxidants can reduce the formation of *trans* fatty acids during thermal processing (Filip et al., 2011). Major *trans* fatty acids identified in the samples were *trans* isomers of linoleic acid, such as trans 9,12-octadecadienoic acid, trans 9, cis 12-octadecadienoic acid, and cis 9, trans 12-octadecadienoic acid.

3.3 Comparison of the effectiveness of passion fruit leaf extract and BHT

According to the present study, the extract at 1000 ppm was more effective than BHT at 200 ppm (maximum permitted level according to the US Food and Drug Administration) in retarding the oxidation of palm oil during heating. The reason for this observation could be the greater antioxidant activity and thermal stability of the plant extract at high temperatures compared with synthetic antioxidants such as BHT and BHA. This has been reported by many other researchers as well. BHA and BHT can be decomposed and volatilized during high-temperature applications (Asadi and Farahmandfar, 2020). The results of the present study are consistent with those of other studies. Nor et al. (2009) studied the antioxidant effect of *Murraya koenigii* leaf extract in palm olein during

deep-frying of French fries at 180 ºC for up to 40 h and reported that the extract at 2000 ppm retarded oil oxidation and deterioration significantly, but less effectively than BHT (200 ppm). Compared with the present study, the passion fruit leaf extract exhibited significantly higher efficiency in retarding oxidation in palm oil during thermal processing. Asadi and Farahmandfar (2020) also reported that *Teucrium polium* leaf extract (200, 600 and 1000 ppm) was effective in retarding oxidation of canola oil during frying at 180 ˚C for 30 h, and the effect of the extract at 1000 ppm was comparable to that of the synthetic antioxidant, BHA (200 ppm). Ethanolic extract of *Cressa cretica* leaves at a concentration of 1000 ppm exhibited higher efficiency in inhibiting oxidation than BHT (200 ppm). Similarly, Asnaashari et al. (2015) reported that Rubus fruticosus leaf extract (1000 ppm) was more effective than BHT, BHA, and other extract concentrations when used in soybean oil during frying. Ali et al. (2026) demonstrated that plant extracts, such as pomegranate flower extract at concentrations of 1,600 and 2,400 ppm, provide significantly better protection in terms of all oxidative parameters than BHT at the maximum legal limit of 200 ppm. This enhanced performance is largely attributed to the greater thermal stability and lower volatility of natural phenolics under extreme processing conditions. As reported by Taghvaei and Jafari (2015), synthetic antioxidants like BHA and BHT are highly susceptible to loss through steam distillation during heating, whereas the bioactive compounds in plant extracts are less volatile and remain active longer within the oil matrix. Furthermore, Zhao et al. (2025) highlighted that synthetic additives such as BHA are prone to thermal decomposition at temperatures of 180 °C, which reduces their antioxidant activity compared to natural compounds. Collectively, these studies suggest that the complex mixture of phytochemicals in natural extracts provides a more resilient, multi-stage defense against the radical-mediated chain reactions that drive oil oxidation.

Table 2: Correlation analysis of the chemical parameters of the negative control during continuous heating of palm oil

Parameter	FA	PV	TPC	CD	CT	p-AV
PV	0.89					
TPC	0.98	0.91				
CD	0.95	0.98	0.95			
CT	0.98	0.93	0.98	0.97		
p-AV	0.98	0.89	0.99	0.94	0.98	
TOTOX	0.94	0.99	0.96	0.99	0.97	0.95

(Values represent the Spearman's correlation coefficient (r) for the linear analysis, Abbreviations: FA – Fatty Acid, PV – Peroxide value, TPC – Total polar compounds, CD – Conjugated dienes, CT – Conjugated trienes, p-AV – p-Anisidine value, TOTOX – Total oxidation)

Table 3: Correlation analysis of the chemical parameters of the positive control during continuous heating of palm oil

Parameter	FA	PV	TPC	CD	CT	p-AV
PV	0.94					
TPC	0.61	0.67				
CD	0.94	0.96	0.72			
CT	0.90	0.96	0.80	0.97		
p-AV	0.95	0.99	0.71	0.96	0.96	
TOTOX	0.94	0.99	0.68	0.96	0.96	0.99

(Values represent the Spearman's correlation coefficient (r) for the linear analysis. Abbreviations: FA – Fatty Acid, PV – Peroxide value, TPC – Total polar compounds, CD – Conjugated dienes, CT – Conjugated trienes, p-AV – p-Anisidine value, TOTOX – Total oxidation)

Table 4: Correlation analysis of the chemical parameters of test samples during continuous heating of palm oil

Parameter	FA	PV	TPC	CD	CT	p-AV
PV	0.97					
TPC	0.90	0.97				
CD	0.91	0.98	0.99			
CT	0.95	0.99	0.98	0.99		
p-AV	0.93	0.99	0.97	0.97	0.99	
TOTOX	0.92	0.97	0.94	0.94	0.97	0.97

(Values represent the Spearman's correlation coefficient (r) for the linear analysis, Abbreviations: FA – Fatty Acid, PV – Peroxide value, TPC – Total polar compounds, CD – Conjugated dienes, CT – Conjugated trienes, p-AV – p-Anisidine value, TOTOX – Total oxidation)

Table 5: Fatty acid composition of negative control samples with heating cycles

Heating cycles	Fatty acids						
	C4-C14	C16	C18	C18:1	C18:2 *t*	C18:2	C18:3
0	4.87±1.12 ^{a}	37.14±2.36 ^{a}	4.03±0.50 ^{a}	40.17±1.21 ^{a}	0.37±0.00 ^{d}	10.37±0.6 ^{a}	0.49±0.05 ^{a}
2	5.55±1.60 ^{a}	40.48±1.50 ^{a}	4.32±0.01 ^{a}	39.90±1.07 ^{a}	0.39±0.01 ^{d}	9.53±0.18 ^{a}	0.52±0.00 ^{a}
4	4.93±0.50 ^{a}	39.47±2.03 ^{a}	4.23±1.20 ^{a}	39.66±2.64 ^{a}	0.37±0.01 ^{d}	9.11±0.85 ^{a}	0.41±0.00 ^{b}
6	8.05±0.90 ^{a}	38.47±1.50 ^{a}	4.15±0.95 ^{a}	38.49±3.10 ^{a}	0.40±0.02 ^{c}	8.44±0.62 ^{b}	0.40±0.00 ^{b}
8	5.68±1.20 ^{a}	40.11±0.85 ^{a}	4.23±1.10 ^{a}	39.58±2.60 ^{a}	0.40±0.01 ^{c}	8.07±0.46 ^{b}	0.41±0.00 ^{b}
10	5.61±0.90 ^{a}	41.93±0.76 ^{a}	4.52±2.00 ^{a}	39.77±1.00 ^{a}	0.42±0.01 ^{c}	7.88±0.55 ^{c}	0.43±0.00 ^{b}
12	5.71±0.80 ^{a}	40.02±1.50 ^{a}	4.29±1.60 ^{a}	38.64±1.50 ^{a}	0.45±0.01 ^{b}	7.19±0.90 ^{c}	0.40±0.06 ^{b}
14	4.96±1.30 ^{a}	42.43±1.00 ^{a}	4.49±0.86 ^{a}	39.54±0.90 ^{a}	0.46±0.01 ^{b}	7.08±0.10 ^{c}	0.43±0.00 ^{b}
16	6.02±0.80 ^{a}	41.62±2.10 ^{a}	4.31±1.30 ^{a}	37.74±3.01 ^{a}	0.45±0.02 ^{b}	6.17±0.2 ^{d}	0.41±0.01 ^{b}
18	5.31±0.81 ^{a}	39.52±1.20 ^{a}	4.14±0.68 ^{a}	36.26±2.02 ^{a}	0.50±0.01 ^{a}	5.88±0.85 ^{d}	0.37±0.01 ^{c}
20	6.99±1.20 ^{a}	39.32±2.10 ^{a}	4.14±0.67 ^{a}	36.02±2.30 ^{a}	0.49±0.02 ^{a}	5.60±0.42 ^{d}	0.35±0.01 ^{c}
22	6.89±0.50 ^{a}	40.63±3.01 ^{a}	4.42±1.30 ^{a}	37.61±2.03 ^{a}	0.50±0.00 ^{a}	5.59±0.18 ^{d}	0.31±0.02 ^{d}
24	5.57±0.82 ^{a}	39.42±2.30 ^{a}	4.23±2.30 ^{a}	34.44±1.57 ^{a}	0.50±0.00 ^{a}	4.77±0.08 ^{e}	0.30±0.07 ^{d}

C18:2t- Trans isomers of linoleic acid (trans 9,12 octadecadienoic acid, trans 9, cis12 octadecadienoic acid, cis 9, trans 12 octadecadienoic acid). Values are means ± standard deviation for triplicate analyses. Means in each column followed by different superscripts letters (a-e) are significantly different (p< 0.05).

Table 6: Fatty acid composition of test samples with heating cycles

Heating cycles	Fatty acids						
	C4-C14	C16	C18	C18:1	C18:2 *t*	C18:2	C18:3
0	4.87±2.12^{a}	37.14±0.36^{a}	4.03±0.02 ^{a}	40.17±0.21^{a}	0.37±0.00^{b}	10.37±0.00^{a}	0.49±0.05^{a}
2	5.71±1.05^{a}	36.19±0.08^{a}	3.79±0.06 ^{a}	38.81±0.08^{a}	0.35±0.01^{b}	10.35±0.03^{a}	0.49±0.01^{a}
4	6.41±0.08^{a}	40.50±0.02^{a}	4.21±0.08 ^{a}	38.96±0.02^{a}	0.35±0.02^{b}	10.15±0.43^{a}	0.51±0.24^{a}
6	5.78±0.55^{a}	32.08±0.58^{a}	3.47±0.09 ^{a}	39.33±0.00^{a}	0.36±0.00^{b}	9.89±0.41^{ab}	0.51±0.00^{a}
8	6.17±0.81^{a}	34.18±0.07^{a}	3.67±0.06 ^{a}	38.46±0.00^{a}	0.35±0.12^{b}	9.71±0.04^{b}	0.52±0.05^{a}
10	5.02±1.21^{a}	37.48±0.04^{a}	3.87±0.04 ^{a}	37.59±0.10^{a}	0.35±0.03^{b}	8.78±0.00^{b}	0.51±0.00^{a}
12	4.24±0.60^{a}	42.60±0.35^{a}	4.37±0.03 ^{a}	39.99±0.19^{a}	0.37±0.00^{b}	8.79±0.05^{b}	0.49±0.01^{a}
14	5.83±0.93^{a}	40.47±0.02^{a}	4.54±0.07 ^{a}	37.85±0.03^{a}	0.37±0.00^{b}	8.49±0.02^{b}	0.45±0.25^{b}
16	6.31±1.05^{a}	42.12±0.04^{a}	4.48±0.01 ^{a}	40.29±0.05^{a}	0.36±0.00^{b}	8.76±0.06^{b}	0.46±0.31^{b}
18	7.56±0.63^{a}	41.62±0.07^{a}	4.32±0.06 ^{a}	38.83±0.07^{a}	0.40±0.01^{a}	7.98±0.03^{c}	0.44±0.45^{b}
20	5.60±0.80^{a}	41.42±0.03^{a}	4.36±0.07 ^{a}	38.11±0.05^{a}	0.39±0.05^{a}	7.90±0.08^{c}	0.40±0.23^{c}
22	7.71±1.07^{a}	42.29±0.07^{a}	4.47±0.02 ^{a}	38.77±1.16^{a}	0.41±0.01^{a}	7.28±0.005^{c}	0.39±0.14^{c}
24	7.61±0.93^{a}	43.60±0.01^{a}	4.57±0.01 ^{a}	38.78±2.14^{a}	0.42±0.03^{a}	6.80±0.02^{d}	0.40±0.00^{c}

C18:2t- Trans isomers of linoleic acid (trans 9,12 octadecadienoic acid, trans 9, cis12 octadecadienoic acid, cis 9, trans 12 octadecadienoic acid). Values are means ± standard deviation for triplicate analyses. Means in each column followed by different superscripts letters (a-e) are significantly different (p< 0.05).

Table 7: Fatty acid composition of positive control samples with heating cycles

Heating cycle	Fatty acids						
	C4:0-C14:0	C16:0	C18:0	C18:1	C18:2 *t*	C18:2	C18:3
0	4.87±0.12^{d}	37.14±3.36^{a}	4.03±1.02^{a}	40.17±2.21^{a}	0.37±0.00^{c}	10.37±0.80^{a}	0.49±0.05^{a}
2	4.57±0.61^{d}	39.79±2.88^{a}	4.07±0.24^{a}	41.12±3.08^{a}	0.37±0.06^{c}	10.90±1.09^{a}	0.51±0.07^{a}
4	4.88±0.31^{d}	37.66±3.56^{a}	4.09±2.05^{a}	40.75±1.08^{a}	0.35±0.01^{cd}	10.11±1.01^{a}	0.49±0.08^{a}
6	5.28±0.05^{c}	39.65±1.15^{a}	3.84±2.13^{a}	37.61±3.06^{a}	0.35±0.05^{cd}	9.07±0.67^{b}	0.49±0.01^{a}
8	5.68±0.15^{b}	39.73±1.90^{a}	3.56±2.02^{a}	38.61±3.29^{a}	0.38±0.00^{c}	7.79±1.83^{b}	0.50±0.02^{a}
10	5.68±061^{b}	38.72±2.07^{a}	4.14±2.07^{a}	38.97±2.14^{a}	0.37±0.00^{c}	8.37±1.44^{b}	0.49±0.02^{a}
12	5.90±0.03^{b}	41.23±1.35^{a}	4.52±2.01^{a}	38.81±3.23^{a}	0.38±0.00^{c}	8.12±0.55^{b}	0.48±0.21^{ab}
14	5.52±0.56^{bc}	40.51±1.11^{a}	4.44±2.41^{a}	39.86±2.31^{a}	0.39±0.00^{c}	6.98±1.18^{c}	0.47±0.02^{b}
16	6.14±0.07^{a}	38.08±0.05^{a}	4.24±2.02^{a}	38.59±2.05^{a}	0.44±0.00^{b}	6.70±0.06^{c}	0.45±0.05^{c}
18	6.19±0.08^{a}	41.88±2.24^{a}	4.35±0.98^{a}	39.68±1.09^{a}	0.45±0.04^{b}	6.62±0.06^{c}	0.45±0.00^{c}
20	5.10±0.17^{c}	37.38±3.35^{a}	3.90±2.87^{a}	38.63±3.81^{a}	0.54±0.01^{a}	5.96±0.09^{d}	0.44±0.01^{c}
22	5.94±0.15^{b}	36.37±2.02^{a}	4.00±1.25^{a}	39.92±2.15^{a}	0.56±0.03^{a}	4.50±0.90^{e}	0.40±0.01^{d}
24	5.62±0.12^{b}	37.92±3.06^{a}	4.08±1.04^{a}	38.31±3.07^{a}	0.57±0.23^{a}	4.43±0.45^{e}	0.37±0.01^{e}

C18:2t- Trans isomers of linoleic acid (trans 9,12 octadecadienoic acid, trans 9, cis12 octadecadienoic acid, cis 9, trans 12 octadecadienoic acid). Values are means ± standard deviation for triplicate analyses. Means in each column followed by different superscript letters (a-e) are significantly different (p< 0.05).

4. Conclusions

Adding fresh passion fruit leaf extract to palm oil reduced free fatty acid content, peroxide value, *p*-anisidine value, CD and CT values, and TOTOX value, and significantly inhibited the formation of total polar compounds compared to the synthetic antioxidant BHT. Major changes occurred in linoleic acid (C118:2) and linolenic acid (C18:3). Both fatty acid contents decreased significantly in all three samples; however, the change was less significant in the test (extract-added oil sample), followed by the positive control. Furthermore, the extract showed the ability to reduce the formation of *trans* fatty acids during heating. Therefore, based on the present study, it is concluded that passion fruit leaf extract can inhibit the oxidation of palm oil to a greater extent than commonly employed synthetic antioxidants, such as BHT, during high-heat processing, despite the poor initial quality of the oil. Since the most of the eateries in Sri Lanka use the poor quality palm oil purchased at low cost, the addition of the plant leaf extract could be an effective solution at least to a particular extent to improve the stability of palm oil during frying as well as to improve the quality of the fried food, especially when the oil is reused for many times as usually practiced in the restaurants in Sri Lanka. However, since there are other factors influencing the oxidation of edible oils during frying, such as the addition of moisture from the food into the oil, further studies need to be carried out to get more accurate results on the effect of the addition of passion fruit leaf extract to palm oil during frying. In conclusion, fresh passion fruit leaf extract could serve as a low-cost source of antioxidants to improve the oxidative stability of palm oil and could replace synthetic antioxidants.

Declaration of conflict of interest

The authors declare no conflicts of interest.

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Author Contribution

I.R.N.D. Jayasekara: Investigation, Methodology, Writing, original draft; S. Sivakanthan**:** Conceptualization, Supervision, Writing, review & editing, Funding acquisition.

Data availability

Data will be made available on request.

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