

Freeze-dried and ultrasonicated methanolic extract of *Fagonia indica* substantially improved oxidative stability and shelf life of sunflower oil: A kinetic study

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Abstract

The present work investigated the ability of freeze-dried and ultrasonicated methanolic *F. indica* extracts to stabilise raw sunflower oil (SFO) compared to butylated hydroxyanisole (BHA). The DPPH activities of the extracts were measured up to 100°C for thermal stability analysis. The total antioxidant power (TAP) assay was used to estimate the antioxidant potential of the extracts. Phytochemical characterisation was performed using ultra-high-performance liquid chromatography-quadrupole time of the plant extract at 600 mg/kg SFO and stored at 30, 40, and 50°C. The peroxide value (PV, in meq O₂/kg) was measured as an oxidative stability parameter to apply the Arrhenius kinetic equation to calculate the activation energy (E_a , in KJ/mol), shelf life (weeks), and reaction rate constant (K). The thermal stability results showed considerable retention of antioxidant activity at 100°C. The reaction for PV formation followed zero-order kinetics, as shown by the regression coefficient (R^2) values, with a direct relationship between peroxide formation and temperature. The blank SFO exhibited an E_a value of 62.04 KJ/mol, which was less than the 102.4 KJ/mol observed for SFO enriched with *F. indica* extract. The K values for blank SFO and extract-enriched SFO were 1.34 and 0.0083 week⁻¹, respectively. Kinetic model-based calculations predicted a shelf life of 6.77 weeks for blank SFO. The shelf life of SFO enriched with *F. indica* extract at 600 mg/kg was 109.39 weeks, significantly higher than that of the blank. The improved oxidative stability was due to the antioxidant potential of the *F. indica* extract. The antioxidant capacity was confirmed using the TAP assay, and metabolite profiling revealed the presence of apigenin derivatives, gallic acid, corilagin, kaempferol, isorhamnetin, and ellagic acid. These findings suggested that *F. indica* could be a promising natural antioxidant candidate for the edible oil industry.

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Introduction

Vegetable oils are widely used in food processing, culinary cooking, and deep frying throughout the globe, and an important component of the human diet. Sunflower oil is an economically

important vegetable cooking oil, and readily available. The global consumption of sunflower oil is estimated to be about 20 million tons in 2024 (Shahbandeh, 2023). Sunflower oil is highly unsaturated, thus susceptible to lipid peroxidation during storage as well as during cooking where

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relatively higher temperatures are involved. Lipid peroxidation continues at ambient storage conditions and exacerbates at high temperatures, especially in food processing which requires elevated temperatures (Raza *et al.*, 2009). The oxidative deterioration of vegetable oils at ambient or accelerated storage conditions proceeds through a complex interplay of factors including photo-oxidation, auto-oxidation, polymerisation, hydrolysis, and oxidation (Loganathan *et al.*, 2022). Lipid oxidation not only produces rancidity but also imparts off-flavour which is highly unacceptable among the consumers. Besides quality deterioration due to oxidation, lipid degradation also results in the production of polar chemical species like free radicals, aldehydes, and ketones which impose serious health-related threats including atherosclerosis, inflammatory problems, arthritis, abnormal digestive tract function, cardiovascular diseases, mutagenic and genotoxic issues, and trigger the incidence of carcinogenesis (Martin *et al.*, 2001; Ganesan *et al.*, 2019; Machado *et al.*, 2023). To enhance the shelf life of vegetable oils, synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butyl hydroquinone (TBHQ) are frequently used, but their safety is not free from questions (Taghvaei and Jafari, 2015). There are many reports on the toxicity of synthetic antioxidants, which is a matter of keen concern among the consumers (Aladedunye, 2014). The BHA, BHT, and TBHQ were reported to exhibit serious health risks including carcinogenesis, apoptosis, genotoxicity, liver damage, and endocrine disruption as shown by various animal model studies (Venkata and Subramanyam, 2016; Yee and Tiu, 2019; Ambreen *et al.*, 2020).

Therefore, the researchers are looking for novel and less toxic or safe antioxidants to be used on commercial scale, especially to stabilise the vegetable oils. The most suitable and workable option is the natural antioxidants present in plants which are safe, cheap, easily available, and more acceptable among consumers (Manassis *et al.*, 2020). The medicinally important plants are well-known sources of natural antioxidants, mainly phenolics and flavonoids, having the strong ability to scavenge free radicals and reactive oxygen species (ROS). Many previous studies have reported the use of plant extracts as an alternative to toxic synthetic antioxidants to stabilise vegetable oils, including sunflower oil. The results exhibited promising antioxidant activities of plant

extracts to enhance the shelf life and oxidative stability of vegetable oils (Raza *et al.*, 2014; Ghendov-Mosanu *et al.*, 2023; Rizwan *et al.*, 2025). However, it has been observed that plant extracts lose their antioxidant potential with the passage of time or when treated at higher temperatures necessary for cooking or frying. The most important aspect is the prediction of the shelf life of vegetable oils for how much time a specific plant extract can hold its antioxidant potential (Manzocco *et al.*, 2016; Singh *et al.*, 2024). The synergistic interplay of natural antioxidants in plant extract with free radicals is a very important phenomenon and considered as the most vital aspect for the oxidative stability of vegetable oils (Le Tan *et al.*, 2025). A recent study reported the improvement in oxidative stability of sunflower oil enriched with saffron by-products at accelerated storage of 60°C for 12-week period. The enhancement of oxidative stability was linked with the high polyphenolic contents in saffron by-product, but the aspect of phytochemical profiling and shelf-life prediction was missing (Ahmed *et al.*, 2025). There is very limited research on kinetic model-based shelf-life prediction of edible oils enriched with plant extracts especially in the context of Pakistan where a huge revenue is paid on the import of synthetic antioxidants. Kinetic study is a requisite tool to determine the shelf life, oxidative stability, activation energy, and Q₁₀ factor of vegetable oils. Using chemical kinetics-based data to predict long-lasting antioxidant properties of plant extracts in terms of shelf life can be completed in shorter experiment period (Upadhyay and Mishra, 2015; Farhoosh, 2022; Dedevas, 2024).

Fagonia indica is a member of the family Zygophyllaceae and a valuable medicinal plant which is locally known as *dhamasa* or *sachi booti* in Pakistan. The *F. indica* is used to treat various ailments exhibiting antidiabetic, anticancer, anti-leishmanial, antipyretic, anti-inflammatory, laxative, gastroprotective, hepatoprotective, and possess antioxidant effects (Ali and Khan, 2021; Gishkori *et al.*, 2024).

The present work was an extension of a previous work to evaluate *F. indica* extract for the enhancement of oxidative stability of sunflower oil. The previously published research highlighted that methanol-based extraction from *F. indica* yielded 24.18 ± 0.36% (240.18 ± 0.36 mg/g extract) extract yield, relatively higher than ethanol, chloroform, *n*-hexane, and ethyl acetate. The methanolic extract

contained an adequate amount of total phenolic contents (184.17 ± 3.28 mg/g of extract in gallic acid equivalent) and total flavonoid contents (102.04 ± 1.02 mg/g of extract in rutin equivalent). The study also reported a DPPH scavenging of $94.84 \pm 0.28\%$ and FRAP value of 303.55 ± 5.05 TE.mM/mL. Further, the inhibition of sunflower oil oxidation based upon peroxide value (PV), free fatty acids (FFA), iodine value (IV), and conjugated dienes and trienes were also observed for sunflower oil added with the extract (Wadhaya *et al.*, 2023). These previous findings served as an impetus to predict the shelf life of sunflower oil with kinetic application using *F. indica* extract. The antioxidant strength, medicinal importance, and potential to improve oxidative stability of vegetable oil by *F. indica* provided strong impulse to execute its further utilisation for sunflower oil stabilisation at various temperatures to predict shelf life before going into commercial aspects. There was also a need to perform metabolite profiling for identification of the important phytochemicals which might be responsible for antioxidant potential of *F. indica*.

The sunflower oil was selected due to its high degree of unsaturation and wide use in the food industry. The peroxide-based oxidative deterioration was utilised to apply kinetic models to predict shelf life, activation energy, and Q_{10} factor to establish its potential as a considerable antioxidant candidate to stabilise the sunflower oil by replacing the commercially available harmful synthetic antioxidants.

Materials and methods

Chemicals and reagents

All the chemicals and reagents used in the present work were of analytical grade. Iodine solution, glacial acetic acid, chloroform, $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, methanol, and starch indicator were the major chemicals used in the present work.

Plant material collection and processing

The plant material was collected from the Dina area of District Jhelum, Punjab, Pakistan, being the natural habitat of *F. indica*. The plant material was immediately quenched in liquid nitrogen and subjected to grinding with continuous supply of liquid nitrogen. The powdery plant material was transferred to freeze dryer (Christ Alpha 1-4 LD, Germany) and freeze drying was conducted for 48 h at -68°C . The

freeze-dried plant material was stored at low temperature in freezer until further use. Freeze drying or lyophilisation has been reported as a highly efficient technique for removal of moisture for longer availability of secondary metabolites of plants (Raza *et al.*, 2020).

Extract preparation

The freeze-dried plant material was suspended in pure methanol and incubated in the dark for 24 h followed by orbital shaking for a further 24 h. The resultant mixture was ultrasonicated at Soniprep 150 using ice cold conditions for maximum recovery of metabolites from plant matrix. Following ultrasonication, the suspended debris was removed through filtration, and the extra solvent was removed by rotary evaporation under vacuum to obtain the extract. The extract yield of 240.18 ± 0.36 mg/g extract was calculated for methanolic extract.

Total antioxidant power (TAP) assay

The total antioxidant capacity of plant extract was determined by TAP assay using previously reported method with slight modifications using ascorbic acid as standard (Shon *et al.*, 2003). The reagent solution was formed using 0.6 M H_2SO_4 , 4 mM $(\text{NH}_4)_2\text{MoO}_4$, and 28 mM Na_3PO_4 . First, 250 mg of extract was weighed and added with 5 mL of reagent solution. A blank sample was also prepared under the same conditions. The sample and blank were then heated in water bath for 90 min at 95°C . The sample was cooled to 25°C and absorbance was read at 695 nm. The results were expressed ascorbic acid equivalents per gram of extract (AE/gE).

Thermal stability of extract

The thermal stability of plant extract was determined in terms of antioxidant activity loss with respect to temperature. The DPPH radical scavenging assay was used for the purpose. The IC_{50} value of extract was measured by preparing the extract concentrations (50 - 250 μg) in 1 mL of methanol for each concentration followed by mixing with DPPH solution. The thermal stability in terms of DPPH activity of plant extract (200 μg) was measured at 25, 50, 75, and 100°C , respectively, in comparison with BHA as standard and gallic acid using established methods. Briefly, the plant extracts were mixed with freshly prepared methanolic DPPH reagent solution with continuous shaking and incubated at 25°C in the dark for 25 min. The absorbance was read at 517 nm,

and Eq. 1 was used for calculating the scavenging % (Mensor *et al.*, 2001; Arabshahi-D *et al.*, 2007).

$$DPPH\ Scavenging\ \% = \left(\frac{Abs\ of\ B - Abs\ of\ S}{Abs\ of\ B} \right) \times 100 \quad (Eq. 1)$$

where, Abs = absorbance, B = blank or control, and S = sample. The IC₅₀ value of extract was computed from the straight-line equation ($Y = 0.192X + 43.46$ with R^2 value of 0.979) and IC₅₀ value of BHA was calculated from $Y = 0.214x + 45.4$ with R^2 value of 0.988.

UHPLC-QTOF-MS/MS analysis of plant extract

The methanolic plant extract of *F. indica* were dissolved in suitable solvent and the resultant suspension was filtered on polytetrafluoroethylene (PTFE) filter having pore size of 0.45 μ m. Following filtration, the samples were introduced to Ion Trap Hybrid Quadrupole-LC-MS/MS (AB Sciex 5600-1 equipped with Eksigent UHPLC) using scanning range of 50 - 1200 m/z for MS/MS. The negative ionisation mode was utilised with 20 μ L injection volume on Thermo Hypersil Gold (100 mm \times 2.1 mm \times 3 μ m) column. The data interpretation for computation of results, Sciexpeak views 2.1 software, ACD labs MS fragmenter software, and ChemSpider database, were used. For the compounds' identification, the fragmentation patterns were also studied manually (Raza *et al.*, 2020).

Kinetic study of sunflower oil oxidation

The refined, bleached, and deodorised (RBD) sunflower oil (SFO) was selected as substrate to assess the impact of plant extracts on the shelf life and kinetic parameters regarding oxidative stability. The sunflower oil was selected due to its high unsaturation and wide use in culinary works. The oxidative deterioration with respect to temperature in relation to heating time was determined by the Rancimat method, and peroxide value (PV) was taken as a core indicator for oxidation phenomenon. SFO samples were stored at three levels of temperatures *i.e.* 30, 40, and 50°C for 7 w. An additional SFO as blank also went through the same protocol. Each SFO sample contained plant extract at concentration of 600 mg/kg of oil except blank. The PV (meq of O₂/kg of SFO) was monitored after every week.

The PV was determined according to AOCS method Cd 8-53 (AOCS, 1998). Firstly, about 5 g of

SFO was taken in iodine flask containing 250 mL iodine solution. After mixing the SFO with iodine solution, glacial acetic acid and chloroform were mixed in 3:2 v/v to form a homogenous mixture. About 30 mL of this mixture was taken and mixed with the SFO previously mixed with iodine solution. Then, 1 mL of the resultant mixture was taken and diluted with 30 mL of distilled water. The free iodine was titrated against the 0.01 N Na₂S₂O₃.5H₂O in the presence of starch solution indicator until the appearance of white colour. The PV was calculated using Eq. 2:

$$PV\ in\ meq/kg = \frac{Vol\ of\ Sod.\ thiosulphate \times N \times 1000}{W\ to\ f\ Sample\ in\ g} \quad (Eq. 2)$$

where, N = normality of Na₂S₂O₃.5H₂O.

The previously reported well established method for kinetic study was used (Darmawan *et al.*, 2023). The PV (meq O₂/kg of oil) obtained through the procedure was plotted against treatment time (7 w). The slopes of developed equations were utilised to obtain the lnK_{pv}, simply by taking natural log of obtained PV, as shown in Eq. 3:

$$\ln K_{pv} = \ln p_v \quad (Eq. 3)$$

The lnK_{pv} obtained from previous step were plotted against inverse of Kelvin temperature (1/T) to apply Arrhenius type plot. The kinetic energies were calculated using Arrhenius plot (lnK_{pv} vs 1/T). The slope of the Arrhenius plot indicated the activation energy values and intercept represented the lnK_A (Matveeva *et al.*, 2024). The R^2 values were also determined to find the most suited model or equation for kinetics, as shown in Eq. 4:

$$\ln K = \ln K_A - EA/R \left(\frac{1}{T} \right) \quad (Eq. 4)$$

where, K = constant rate, E_a = activation energy in J/mol, R = universal gas constant (8.314 Jmol⁻¹K⁻¹), and T = temperature in Kelvin. The rate constant for PV at 25°C was then determined using Eq. 5:

$$k = e^{[Intercept - \frac{(Slope)1}{T}]} \quad (Eq. 5)$$

The shelf life was determined using Eq. 6:

$$Ts = \frac{Cs - Co}{K} \quad (Eq. 6)$$

where, T_s = shelf life, C_s = standard PV, C_o = initial PV at the start of reaction or zero time, K = rate constant in unit of week^{-1} .

The Q_{10} was determined using Eq. 7:

$$Q_{10} = Cf/Co \left(\frac{10}{T_2 - T_1} \right) \quad (\text{Eq. 7})$$

where, C_f = final PV at the end of reaction time, and C_o = initial or zero-time value of PV.

Results and discussion

The antioxidant activity and thermal stability of plant extract was an important aspect required to stabilise the lipids and lipid-containing foods. The antioxidant potential and thermal resistance exhibited by plant extract made them suitable for their utilisation in edible oils to impart oxidative stability, especially where long-term storage or thermal exposure like heating or frying was involved (Taghvaei and Jafari, 2015). The findings of the present work indicated that a reasonable DPPH radical scavenging was observed for *F. indica* extract even at 100°C . The DPPH activity revealed that the plant extract was adequately stable at high temperature, making it suitable for edible oil stabilisation. However, the BHA was more stable and showed retention of 65.15% DPPH scavenging potential as compared with plant extract having DPPH radical scavenging value of 40.11%. The DPPH activity of *F. indica* extract remained in between the BHA and gallic acid (Figure 1).

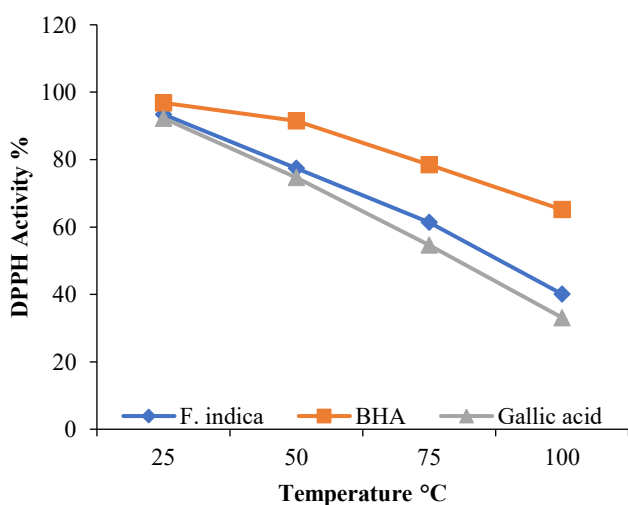


Figure 1. DPPH activity of *F. indica* extract in relation to temperature.

Beyond thermal stability, the IC_{50} value of plant extract and BHA were calculated and found to be 34.06 and 21.49 $\mu\text{g/mL}$, respectively. The IC_{50} value of less than 100 $\mu\text{g/mL}$ for DPPH antioxidant activity was categorised as strong, and less than 50 $\mu\text{g/mL}$ as very strong (Jumina *et al.*, 2019). The IC_{50} value of *F. indica* extract corresponded to very strong antioxidant activity. The TAP assay values of plant extract and ascorbic acid as standard compound are reported in Figure 2.

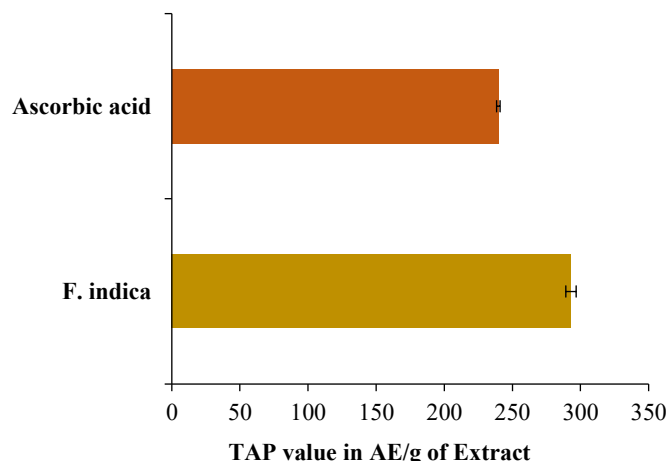


Figure 2. TAP comparison of *F. indica* extract and ascorbic acid.

The TAP value of *F. indica* came out to be 293.0 ± 3.77 AE/gE which was higher than ascorbic acid, for which the value was 239.66 ± 1.25 AE/gE. The TAP assay involved the reduction of Mo^{+6} to Mo^{+5} by metabolites present in plant extract resulting in green colour complex formation having absorbance wavelength of 695 nm (Farooq *et al.*, 2020). The TAP value of plant extract was evident in the presence of secondary metabolites having antioxidant potential, which was confirmed by UHPLC-QTOF-MS/MS analysis. The metabolite profiling revealed the presence of some high value compounds as shown in Table 1.

The fragmentation patterns of the compounds were also studied for the confirmation of metabolites. Many identified metabolites were reported to exhibit strong antioxidant activities (Raza *et al.*, 2020; Farooq *et al.*, 2020). The UHPLC-QTOF-MS/MS-based metabolite identification was reported as an excellent technique for complex plant extract analysis having a large number of compounds contributing to the antioxidant activity of extract (Wong *et al.*, 2024). Based upon the results of thermal stability in terms of

Table 1. UHPLC-QTOF-MS/MS-based identification of secondary metabolites in methanolic extract of *F. indica*.

Sr. No.	Compound	Retention time (min)	Molecular ion peak (m/z)	Major fragments (m/z)	Molecular formula
1	Apigenin-C-hexoside-C-hexoside	6.94	593.15	503.12, 473.10, 383.07, 353.06	C ₂₀ H ₃₄ O ₂₀
2	Gallic acid	8.54	169	125, 124	C ₇ H ₆ O ₅
3	Gibberellin A4	9.6	331	316, 287, 213, 199, 190, 163, 161, 148	C ₁₉ H ₂₄ O ₅
4	Corilagin	10.2	633	615, 571, 481, 419, 341, 329, 301, 299, 275, 169	C ₂₀ H ₂₆ O ₂₃
5	Kaempferol	12.7	285	205, 206, 189, 79.9	C ₁₅ H ₁₀ O ₆
6	Isorhamnetin	16.1	315	300, 271, 227, 151, 164, 148	C ₁₆ H ₁₂ O ₇
7	Ellagic acid	17.932	301	300, 299, 283, 229, 173, 96	C ₁₄ H ₆ O ₈

antioxidant activity and the presence of important metabolites, the extract was used to stabilise the SFO samples for kinetic model application. The kinetic studies were based upon some of the sensory markers for oxidative damage. Measurement of such sensory oxidative parameter or marker was required to proceed for application of kinetic models. In the present work, the PV was used as a sensory parameter for kinetic model application. PV, being an important rancidity parameter was considered for determination of oxidative damage to edible oils and for the application of kinetic models (Elhussain *et al.*, 2018; Zhang *et al.*, 2021a). The PV was measured at three

different temperatures for seven weeks. The PV against time plots were drawn accordingly. The slopes of the plots were used as K_{pv} and the natural logarithm of K_{pv} were taken. The $\ln K_{pv}$ values were plotted against $1/T(K)$ and the value of slope indicated the E_a in KJ/mol. The values of the rate constant K were computed from Arrhenius equation, and these K values were used to calculate the shelf life at 25°C (Figure 3).

The R^2 values, K_{pv} , and $\ln K_{pv}$ calculated from the data of blank SFO presented in Figure 3 are presented in Table 2.

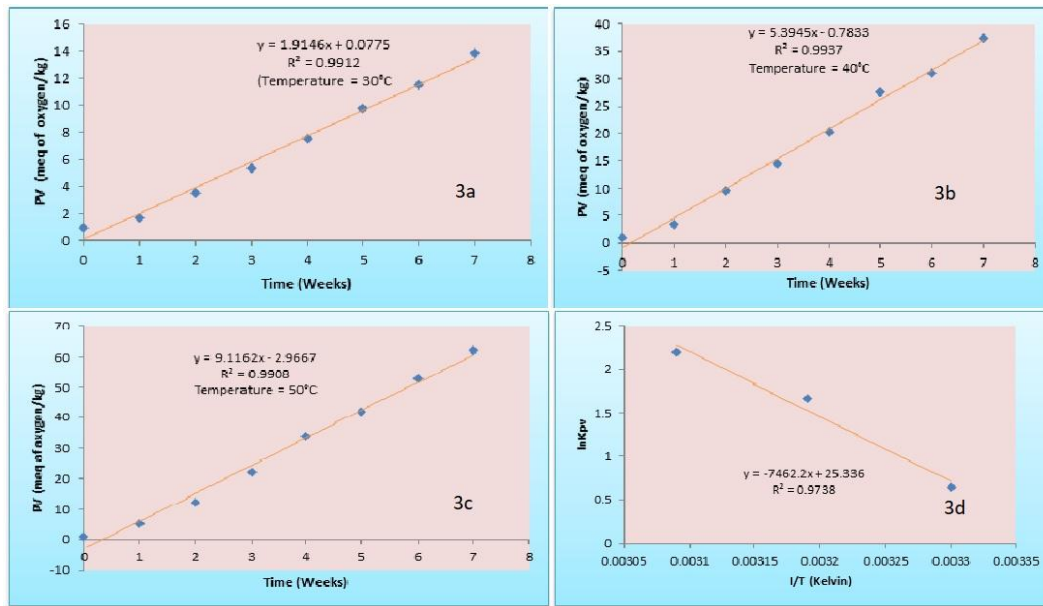


Figure 3. (a), (b), and (c) represent the dynamics of PV (meq O_2 /kg of SFO) for blank with respect to time, and (d) represent the dependency of logarithmic reaction rate on inverse of temperature (K).

Table 2. Kinetic parameters of hydrogen peroxide formation in blank SFO.

Sr. No.	Temperature (°C)	R^2	K_{PV} (meq O_2 /kg of oil)	$\ln K_{PV}$
1	30	0.9912	1.9146	0.64
2	40	0.9937	5.3945	1.68
3	50	0.9908	9.1162	2.2

The slope obtained from the Arrhenius plot (Figure 3d) was used to find out the energy of activation for PV formation for blank SFO using Eq. 8:

$$E_a = \frac{7462.2 \times 8.314}{1000} = 62.04 \text{ KJ/mol} \quad (\text{Eq. 8})$$

Similarly, the rate constant for blank SFO for 25°C was computed from slope and intercept of the linear equation (Figure 2d) using Eq. 9:

$$k = e^{[25.336 - \frac{7462.2}{298K}]} = 1.34 \quad (\text{Eq. 9})$$

The rate constant K value was used to predict the shelf life of blank SFO at 25°C using Eqs. 10 and 11:

$$T_s = \frac{C_t - C_0}{K} \quad (\text{Eq. 10})$$

$$T_s = \frac{10 - 0.92}{1.34} = 6.77 \text{ (Weeks)} \quad (\text{Eq. 11})$$

where, C_t = limit of PV as standard. The PSQC limit of PV is 10 meq/kg of oil. This value was used as reference standard (Mehmood *et al.*, 2012).

The R^2 values, K_{pv} , and $\ln K_{pv}$ calculated from the data of SFO enriched with *F. indica* extract presented in Figure 4 are presented in Table 3.

Based on the data given in Figure 4 and Table 3, the E_a , shelf life, and Q_{10} were calculated for SFO enriched with *F. indica* extract using Eq. 12:

$$E_a = \frac{12322 \times 8.314}{1000} = 102.4 \text{ KJ/mol} \quad (\text{Eq. 12})$$

Similarly, the rate constant for SFO enriched with *F. indica* extract for 25°C was computed from slope and intercept of the linear equation (Figure 4d) using Eq. 13:

$$k = e^{[38.867 - \frac{(12322)}{298K}]} = 0.083 \quad (\text{Eq. 13})$$

The rate constant K value was used to predict the shelf life of SFO enriched with *F. indica* extract at 25°C using Eqs. 14 and 15:

$$T_s = \frac{C_t - C_0}{K} \quad (\text{Eq. 14})$$

$$T_s = \frac{10 - 0.92}{0.083} = 109.39 \text{ (Weeks)} \quad (\text{Eq. 15})$$

The values of regression coefficient, E_a (KJ/mol), rate constant (week^{-1}), shelf life (weeks), and temperature acceleration factor (Q_{10}) are summarised in Table 4.

The results revealed that the PV values increased as a function of temperature, which confirmed zero-order kinetics, and the data complied with Arrhenius model for zero-order kinetics. The regression coefficient (R^2) values were also very close to 1, indicating the suitability or best fitness of zero-order Arrhenius model. Based on obtained data, the values of E_a (KJ/mol) clearly reflected that the blank SFO was more pronounced toward oxidation as compared to SFO enriched with *F. indica* plant extract. The E_a for SFO enriched with plant extract exhibited a higher energy of activation required to proceed for oxidation reaction as compared to blank SFO (Saldaña and Martínez- Monteagudo, 2013;

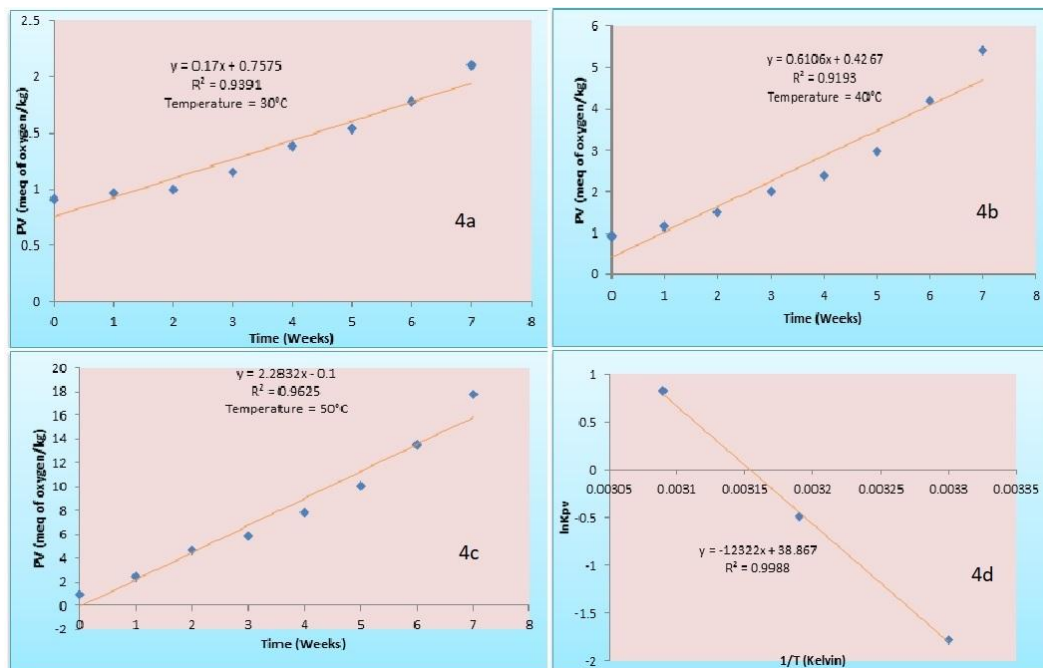


Figure 4. (a), (b), and (c) represent the dynamics of PV (meq O₂/kg of SFO) enriched with *F. indica* against time, and (d) represent the dependency of logarithmic reaction rate on inverse of temperature (K).

Table 3. Kinetic parameters of hydrogen peroxide formation in SFO enriched with *F. indica* extract.

Sr. No.	Temperature (°C)	R^2	K_{PV} (meq O ₂ /kg of oil)	$\ln K_{PV}$
1	30	0.9391	0.17	-1.77
2	40	0.9193	0.6106	-0.48
3	50	0.9625	2.2832	0.825

Table 4. Kinetic parameters from Arrhenius Model for hydrogen peroxide formation in SFO samples.

SFO type	R^2 (regression coefficient)	E_a (KJ/mol)	Rate constant K at 25°C (Week ⁻¹)	Shelf life (Weeks)	Q_{10} (Temperature acceleration factor)
Blank	0.9738	62.04	1.34	6.77	2.17
<i>F. indica</i>	0.9988	102.4	0.0083	109.39	2.93

Darmawan *et al.*, 2023). The constant value for blank SFO was very high as compared to SFO enriched with *F. indica* extract, establishing that the rate of oxidation reaction for PV formation was more vigorous for blank SFO. The most important feature of the present work was the vast difference in the predicted shelf life of blank SFO and SFO enriched with plant extract at 25°C. The extract-enriched SFO exhibited 109.3 weeks shelf life which was much higher than shelf life of blank SFO which was 6.77 weeks only (Table 3). The very low shelf life of blank SFO might be due to the absence of any antioxidant additive and high degree of unsaturation. The dependence of shelf life of SFO on temperature and light exposure remained a matter of keen interest. A study reported shelf life of 9.04 - 10.45 weeks for SFO stored at 40°C (Makhoul *et al.*, 2006); this shelf life might be higher at relatively lower temperature of 25°C. A study conducted in Tanzania on SFO obtained through various extraction methods reported 48 - 72 weeks shelf-life period at room temperature in the dark (Katonge, 2025). The shelf-life prediction of 109.39 weeks for SFO enriched with *F. indica* extract being higher than 72 weeks imparted evidence to the antioxidant role of extract.

The interpretation of kinetic data revealed that an inverse relationship was observed between the rate constant and shelf life, which demonstrated that a shelf-life rate was responsible for low shelf life. The prediction of shelf-life period was based upon the permissible level of PV for edible oils. The delay in lipid peroxidation in terms of PV production for SFO enriched with plant extract was possibly due to the antioxidant nature of the extract. The observation was also confirmed by the findings of DPPH assay performed at various temperatures. The IC₅₀ value of plant extract used for SFO stabilisation provided affirmative evidence of antioxidant potential. The antioxidant potential of extract might be the decisive factor for inhibition of lipid oxidation. The secondary metabolites present in plant extract probably played their role in the complex lipid oxidation process retarding the free radical or reactive species formation, which consequently increased the shelf

life of oil (Yang *et al.*, 2016; Blasi and Cossignani, 2020). It was highly necessary to understand the mechanism of lipid oxidation in vegetable oils. The oxygen molecule reaction with oil proceeded with free radical's production to initiate chain reactions until termination. The thermal degradation of vegetable oils was usually observed when oils were exposed to relatively higher temperature, leading to deterioration of oil quality and sensory attributes. The production of primary oxidation products continued the reactions, resulting in production of secondary oxidation products like aldehydes and ketones imparting off-flavour, rancidity, and toxicity in vegetable oils (Machado *et al.*, 2023). The structural features of secondary metabolites, especially phenolic compounds including phenolic acids and flavonoids, have the ability to donate proton to the free radicals for their stabilisation, thus inhibiting lipid oxidation (Rizwan *et al.*, 2025). The metabolite profiling of *F. indica* extract revealed the presence of gallic acid, quercetin, ellagic acid, apigenin derivative, corilagin, and isorhamnetin. All these compounds were well cited for their antioxidant potential (Ali *et al.*, 2024). Gallic acid was reported to be present in medicinally important plants and considered as an excellent antioxidant especially for lipids due to its unique 3,4,5 trihydroxyl groups contributing to hydrogen donating capability (Molski, 2023). Similarly, a study based on quantum mechanical treatment reported that ellagic acid proton transfer mechanism to stabilise hydroxyl radical (Tošović and Bren, 2020). Besides free radical scavenging, the plant phenolics mechanistically interplayed to absorb singlet and triplet oxygen or by dissolving peroxides (Kedir *et al.*, 2023). It might be inferred that the antioxidant nature of the identified metabolites was the key factor to improve the oxidative stability and shelf life of SFO. The findings from the present work revealed that blank SFO exhibited comparatively low shelf life as compared to previously published research (Upadhyay *et al.*, 2017; Matveeva *et al.*, 2024). However, the use of *F. indica* extract for stabilisation of SFO illustrated a considerable improvement in shelf life and a notable

reduction in rate constant for oxidation reaction. Again, the most probable feature behind the inhibition of lipid oxidation was antioxidant activity of extract interplaying through synergic mechanism. The reported value of Q_{10} for food materials usually ranged from 1 - 3 and higher values were associated with rapid oxidation in fatty foods. The Q_{10} values obtained from the present work were quite reasonable and remained in the promising range when compared with some of the previously published reports (Arabshahi-D *et al.*, 2007; Gandova, 2024). The PV-based kinetic modelling for fats and fatty foods was often used to predict their shelf life using Arrhenius equation at various temperatures and time period (Zhang *et al.*, 2021b; Choosuk *et al.*, 2022). A PV-based kinetic study reported shelf life of 132.7 and 46.97 weeks for sunflower oil enriched with TBHQ and green tea extract, respectively. The shelf-life improvement of SFO in the present work was comparable to TBHQ-stabilised SFO and higher than the green tea extract-stabilised SFO making *F. indica* a better choice. However, PV-based kinetic study accompanied by relatively higher shelf life as compared to *p*-anisidine and Trolox models (Ahmadi *et al.*, 2024). As the PV-based kinetic prediction of shelf life for SFO addressed primary oxidation only; therefore, the shelf-life prediction of SFO might also be accompanied by the secondary oxidation indicators like *p*-anisidine and Trolox for a more rational and realistic approach. Further, microencapsulation techniques should be employed in future studies to enhance the antioxidant availability of *F. indica* extract for food systems. Overall, the findings of the present work provided the information that stabilisation of SFO with *F. indica* extract considerably improved the shelf life of SFO. The inhibition of lipid peroxidation was most probably due to antioxidants and antiradical potential of secondary metabolites present in the plant extract.

Conclusion

The zero-order Arrhenius type kinetic model application to lipid oxidation in terms of PV variation provided useful set of information about kinetic energy and shelf-life period of SFO. The PV value changes were in direct relation to treatment or storage temperature. The comparison of blank SFO and *F. indica* extract-enriched SFO demonstrated marked improvement in shelf life providing a potential indigenous source to reduce or mitigate the use of

synthetic antioxidant in edible oil industry. The antioxidant interplay of identified metabolites was the most probable mechanism for improving the shelf life of SFO. However, secondary oxidation products should also be considered for future studies to predict more reliable and rational shelf life of vegetable oils using *F. indica*.

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