Brazilian Journal of Chemical Engineering (2022) 39:527–538
https://doi.org/10.1007/s43153-021-00150-1

ORIGINAL PAPER

# Bioactives from pomegranate peel and moringa leaves as natural antioxidants for stability of edible oil blends

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Received: 17 February 2021 / Revised: 25 August 2021 / Accepted: 29 August 2021 / Published online: 6 September 2021


## Abstract

The present study investigates oxidative stability using the Rancimat and the Schaal oven test of blends of various edible oils with medium chain triacylglycerols containing natural antioxidants extracted from moringa leaves and pomegranate peel. The moringa leaves and pomegranate peel extract yield was 19.8% and 44.6% respectively whereas the antioxidant activity quantified using the DPPH radical scavenging activity was 95.1% and 92.5% respectively at loading of 900 ppm of extract. The natural extracts also demonstrated efficient antimicrobial activity. The stability of blends was assessed in terms of peroxide value, and using the shall oven test and rancimat study. It was clearly demonstrated that the natural extracts retarded the rate of oxidation in the blends at ambient temperature and also at elevated temperature. In addition, the induction time for rancimat study was higher in the presence of natural extracts demonstrating enhanced shelf life of the blends. Overall, it was clearly demonstrated that natural antioxidants could substitute harmful and carcinogenic chemical antioxidants.

**Keywords** Pomegranate peel · Moringa leaves · Natural antioxidants · Stability · Edible oil blends

## Abbreviations

| | |
|---|---|
| BHA | Butylated hydroxyanisole |
| BHT | Butylated hydroxytoluene |
| DPPH | 2,2-Diphenyl-1-picrylhydrazyl |
| IT | Induction time |
| LCT | Long-chain triacylglycerols |
| MCT | Medium chain triacylglycerols |
| MOL | *Moringa oleifera* Leaves |
| MOLE | *Moringa oleifera* Leaves extract |
| PP | Pomegranate peel |
| PPE | Pomegranate peel extract |
| PV | Peroxide value |
| TBHQ | Tertiary butylhydroquinone |

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## Introduction

Oils and fats are an important part of human diet as they provide energy, essential fatty acids and are carriers of vitamins required for normal functioning of our body. Fats also provide flavour and texture improving the sensory quality of the food products. Almost all long-chain triacylglycerols (LCT) which are edible oils such as rice bran oil, olive and soybean oil possess monounsaturated and polyunsaturated fatty acids with tocopherols. High consumption of LCT requires various steps for digestion and if taken in excess than that consumed by body in terms of energy consumption, the excess LCT is likely to be stored as fat causing various health hazards such as heart attack, strokes and diabetes. Medium chain triacylglycerols (MCT) are the oils with medium chain fatty acids. MCT rapidly solubilize, can be digested easily without real need of bile or pancreatic enzymes (Shah and Limketkai 2017) and are considered a quick source of energy, thus offering health benefits. However, MCT lacks essential fatty acids and tocopherols. Considering these issues, blends of MCT and LCT demonstrate value addition in terms of supply of energy and essential fatty acids to the consumers. Incorporation of MCT also gives benefits such as provision of quick energy without much weight gain. Based on this important facet, MCT has numerous pharmaceutical and nutraceutical applications. The application is

favored by the key characteristic of MCT in terms of quick consumption without much of accumulation. MCT can be particularly useful for the ketogenic diets.

Prevention of blends from oxidation is one of the important considerations during the storage of blends. Oxidation of oil blends lowers the quality by changing the physical, chemical, sensory, and nutritional properties of oils. Unsaturated fatty acids are highly susceptible to oxidation resulting in undesirable changes in flavour and other changes unappealing to consumers (Ahmad and Beg 2001; Alasalvar et al. 2001). The oxidation of oils also causes negative impact on human health. The prolonged consumption of rancid oils also leads to development of cancer and coronary heart disease (Nadeem et al. 2013). Thus, it becomes necessary to prevent the oxidation of the oils either in the native form or the blends depending on the specific application.

Lipid oxidation in food can be prevented by addition of antioxidants. An antioxidant is generally the substance having ability to significantly prevent or delay the oxidation process (Halliwell 1995) even at small concentrations. The chemical antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are widely used to prevent oxidation of oils and oil containing products. In current scenario, consumers prefer natural products due to the toxicological effect of chemical products and thus search of natural antioxidants in fruits, leaves and vegetables that can be used to prevent oxidative process has been on significant rise in recent years (Ben et al. 1996). Jeong et al. (2004) reported that natural antioxidants from plants showed higher or comparable antioxidant activity. Hes et al. (2017) also reported the effective use of antioxidants obtained from plant extracts on lipid stability and changes in protein nutritional value of frozen-stored meat products. Typically, polyphenols and flavanoids drive the antioxidant activity (Bonilla et al. 2011) and these natural antioxidants can be more efficient and safer than synthetic antioxidants. For example, Maqsood et al. (2012) reported that natural antioxidants stabilized fish oil more effectively compared to synthetic antioxidants. It is an added advantage and a sustainable approach if the natural antioxidants are obtained from sustainable sources such as fruit or vegetable or plant wastes as this also partially solves the problem of utilization of the solid waste material. Das et al. (2018) indeed reported one such approaches of using marine industry wastes for isolation of collagen hydrolysate and its subsequent application as peroxide inhibition agents in lipid-based food.

The peels of fruits are considered as waste but they are also a rich source of antioxidants (Balasundram et al. 2006). The fruits wastes are a cheap source of many value-added constituents including antioxidants such as polyphenols and can be considerably used for economic benefit in food processing (Singh and Immanuel 2014). The fruit peels are also abundant in dietary fibers and phenolics which show antioxidants, antimutagenic, cardio preventive, antibacterial and antiviral activities (Adams et al. 2006). As a specific example, pomegranate peel (PP) extract has been reported to inhibit cell growth giving effects in prevention of cancer (Zarfeshany et al. 2014). Pomegranate peel shows high antioxidant activity due to presence of phenolic acids such as ellagic acid, gallic acid etc. and flavonoids like catechin, epicatechin, quercetin, rutin etc. as well as tannins such as punicalin and pedunculagin (Yasoubi et al. 2007; Gil et al. 2000; Tanaka et al. 1990; Elfalleh et al. 2012).

*Moringa oleifera Lam* is another important species as all parts of the plant can be useful in various health applications (Fahey 2005; Mbikay 2012). *Moringa oleifera* leaves (MOL) possesses health-promoting phytochemicals. *Moringa oleifera* leaves have very high nutritional value and are rich source of vitamins, minerals and essential amino acids (Fahey, 2005). Moringa leaves possess high amount of flavonoid compounds such as rutin, kaempferol myricetin, quercetin, and isorhamnetin (Madukwe et al. 2013) which can delay the oxidation process based on the distinctive structural components (Sreelatha and Padma 2009). Mature *Moringa oleiferia* leaves have very strong antioxidant activity due to the presence of phenolic compounds (Das et al. 2012). The leaves of *Moringa oleifera* were indeed reported to be effective in the stabilization of sunflower oil (Anwar et al. 2007).

The aim of the current study was to evaluate the effectiveness of extracts of both moringa leaves (MOL) and pomegranate peel (PP) to prevent the oxidation of edible oil blends (LCT with MCT). Comparison of the antioxidant activity with commercial antioxidants such as BHA and BHT has also been presented. The work is novel as earlier studies have dealt with single edible oil but the present work deals with use of different edible oils and also in combination with MCT so as to give advantages of the blends of LCT and MCT. The work also allows comparison of the effectiveness of use of MOL and PPE as natural antioxidants for a variety of edible oils, which is not seen in the open literature. Comparison with chemical anitoxidants also enables demonstrating the effectiveness of the natural antioxidants obtained from sustainable sources as a potential alternative to the harmful chemical antioxidants.

## Materials and methods

### Materials

Edible oils such as olive oil, flaxseed oil, fish oil, sunflower oil, soybean oil, and moringa oil considered as mainly LCT were obtained from Earth Expo Pvt. Ltd., Gujarat, India. Moringa *oleifera* leaves (MOL) and Pomegranate peel (PP) were procured from local market of Matunga, Mumbai. BHT

and BHA were procured from Hi-media, Mumbai, India. Sodium thiosulphate, potassium iodide, DPPH solution, ethanol and starch were procured from S.D Fine-Chem Pvt. Ltd., Mumbai, India.

## Methods

### Extraction of bioactives from Moringa *oleifera* leaves (MOL) and pomegranate peel (PP)

Moringa leaves and pomegranate peel were used as source of natural antioxidants. The main types of bioactives present in MOL are flavonoids, vitamins, phenolic acids, tannins, isothiocyanates, and saponins whereas those present in PPE are flavonoids, phenolic acids, and hydrolysable tannins. For the preparation of extract, fresh moringa leaves and pomegranate peel were washed and blanched with 0.2% potassium metabisulphate followed by oven drying at 50 °C for 8 h (Ben Nasr et al. 1996). The dried leaves and peels were powdered in the blender and further extraction of bioactives was done. 25 g of MOL and PP powder were separately added to 250 ml beaker consisting of 90% ethanol and 10% water. The photographic representation of the obtained solutions during the extraction has been represented in Fig. 1A, B for moringa leaves and pomegranate peel respectively. The beaker was sealed tightly with aluminum foil so as to avoid loss of any volatiles. A magnetic stirrer operated at 150 rpm for 48 h was used to achieve uniform mixing of the contents.

Extraction yield of MOL and PP was calculated using following formula:

$$\text{Extraction yield } (\%) = (\text{weight of the residue}) / (\text{total weight of the peel powder}) \times 100$$

where the residue considered for the analysis is after the evaporation of solvent.

After the extraction, the solution was allowed to settle for ease of separation in the subsequent step. The separated layer was filtered and subsequently ethanol removed by rota-Vacuum. The extract was used for further studies by adding to the blends of MCT (tricaprylin freshly synthesized in the laboratory from reaction of caprylic acid with glycerol: for detailed synthesis process, earlier work of More et al. (2017) can be referred) and LCT (edible oils such as olive oil, flaxseed oil, fish oil, sunflower oil, soybean oil, and moringa oil) in the proportion of 20:80 (MCT:LCT by mass). Extracts were added at three different concentrations of 300 ppm, 600 ppm and 900 ppm. A control sample i.e., without any antioxidant and two samples containing 100 ppm of BHA and 100 ppm BHQ respectively were also used for comparison. All the samples

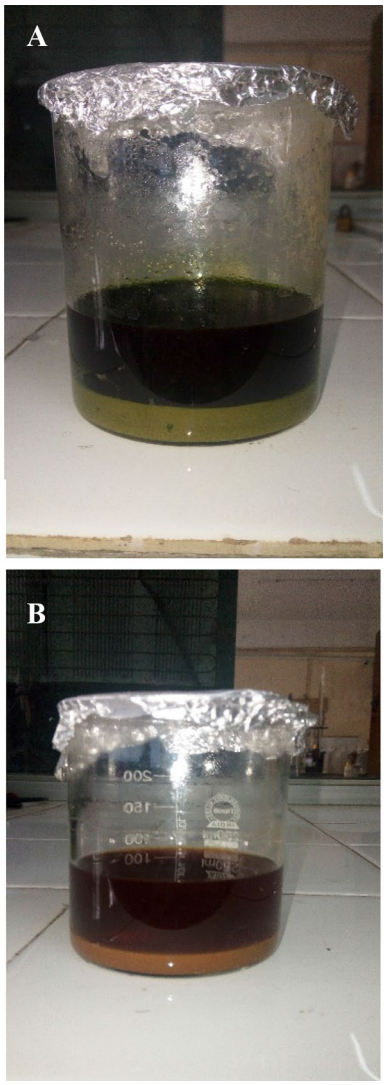


**Fig. 1** Extraction of Bioactives from Sustainable sources (**A**) moringa leaves (**B**) pomegranate peel

were kept at room temperature for 2 months with adequate protection from light and microsamples were withdrawn regularly for analysis of the oxidative stability.

### Evaluation of antioxidant activity

The antioxidant activity of moringa leaves and pomegranate peel in the oil blends was measured by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay (Jadid et al. 2017). Different concentrations of solutions were prepared for both MOL and PP extracts at varying concentrations over the range of 100 ppm to 900 ppm. The ethanolic solution of DPPH was added to the prepared sample and standard solutions. All the solutions were kept in dark at room temperature for 30 min. The absorbance was subsequently measured at 517 nm for the analysis. The absorbance of DPPH without antioxidant was

used as control. The antioxidant activity (AA) was determined using the following formula:

$$AA\% = \left[(\text{Absorbance control} - \text{Absorbance sample}) \times 100/\text{Absorbance control}\right]$$

The measurements for antioxidant activity based on the DPPH assay were performed with proper precautions and also repeated to check the reproducibility. The experiments were performed in triplicate, and the results are expressed as the mean. Only when desired reproducibility was obtained, the data was considered as final.

### Evaluation of antimicrobial activity

The antimicrobial activity was measured by studying inhibition exhibited by the *Moringa oleifera* leaves extract (MOLE) and Pomegranate peel extract (PPE) in the blend samples on the agar plates containing microbes (Balouiri et al. 2015). Gram-positive strains of *Staphylococcus aureus, Bacillus cereus*, and gram-negative strains of *Escherichia coli* and *Pseudomonas aeruginosa* were used in the present study. The initial selection of the microbes was on the basis of having both representations from gram-positive and gram-negative strains and subsequently considering the applications for human consumptions where these microorganisms have shown significant problems (Khan et al. 2011; Pal et al. 1995; Devendra et al. 2011). The antimicrobial assay was done by streaking methods and the growth of microbes was tested. Different concentrations of both extracts as 300 ppm, 600 ppm and 900 ppm were used for studying the inhibition zone. Each microbial strain was uniformly spread over the solid media plate. The surface was streaked with samples containing different concentrations of MOLE and PPE. The plates were incubated at 37 °C for a time period of 24–48 h. Based on the measurements of the diameter of the clear zone shown on plates (expressed in mm) in multiple runs, the antibacterial activity was quantified. Adequate precautions were indeed taken to ensure the correctness of the trends for this widely accepted method based on streaking.

### Determination of oxidative stability using the Rancimat test

The Rancimat test (Maszewska et al. 2018; Tinello et al. 2020) was performed using automated Metrohm Rancimat equipment (model 892) set at a constant heating block temperature of 120 °C. 5 g of blend of oil containing antioxidant and control without antioxidants were added to rancimat tubes. 60 mL of deionized water was added to the measuring vessels which were connected to the instrument by electrodes. The tubes containing samples were sealed properly and then connected to measuring vessels and instrument. The gas flow was maintained at 20 L/h. The induction time was quantified as the difference in the time from start of procedure till the sample turns rancid as automatically recorded by the instrument. The measurements for the induction time were always repeated to check the reproducibility and it was confirmed that the obtained trends are definitely not within the limits of experimental errors, establishing the correctness of the trends.

### Determination of oxidative stability using the Schaal oven test

The Schaal oven test (Maszewska et al. 2018) was carried out to determine oxidative stability of the blends at accelerated storage conditions. The blends of oils with antioxidants and control samples were kept in airtight glass bottles and placed in electric hot air oven at room temperature and 60 °C for one month. The analysis samples were withdrawn after regular intervals of 5 days and oxidative disintegration was studied by measuring peroxide value of the samples. 5 g oil sample was taken and 30 ml of acetic acid: chloroform solution (60% of acetic acid) and 0.5 mL saturated KI were added to the oil sample. The mixture was kept in dark for 1 min and 30 ml distilled water was added to the flask and subsequently titrated against 0.01 N Sodium thiosulphate using starch as indicator. The peroxide value was calculated using formula:

$$\text{Peroxide value} = (\text{sample} - \text{blank}) \times 1000 \times \text{normality}/\text{wt. of sample}$$

### Total polyphenols content in sample:

Folin-Ciocalteu reagent was used for determination of total polyphenols in sample. Folin-Ciocalteu reagent was diluted with distilled water (1:10). 0.5 ml of diluted Folin-Ciocalteu reagent was mixed with 0.1 ml of methanolic extract of sample. The mixture was allowed to stand at room temperature for 5 min. To this mixture, 1.5 ml of sodium carbonate solution was added, followed by addition of 10 ml distilled water and absorbance was measured at 735 nm after 20 min incubation with agitation at room temperature. Results were expressed in mg of gallic acid equivalents (GAE) per 100 g of sample.

### Reproducibility of results

All the experiments were repeated at least two times to check the reproducible nature of the obtained results. It was observed that the results were indeed reproducible and as per the analysis performed using the MS Excel package, the obtained variations in the experimental results were ± 2% of the reported

average values. This established that the experimental data was reproducible.

## Results and discussion

### Extract yields and effect of extract on antioxidant activity

The extract yield was observed to be 19.8% for MOL and 44.6% for PP under identical extraction conditions. The higher yields for PP can be attributed to the higher affinity of the active ingredients present in the PP as substantial amounts of phenolic compounds, such as hydrolysable tannins, flavonoids (anthocyanins and catechins) and nutrients towards ethanol used as the extraction solvent. Similar trends in terms of higher yields for PP has been reported in the literature. For example, Iqbal et al. (2008) reported 29.16% as the yield of pomegranate peel whereas Sultana et al. (2009) reported yield of 17.23% from ethanolic extract of moringa leaves. The obtained yield of pomegranate peel extract was also reported to be 48.2% by Zaki et al. (2015). As per literature, higher amount of polyphenols are extracted using combination medium of water and alcohol (also used in the current work) rather than individual extraction using water or alcohol (Singh and Immanuel 2014). The extraction method mainly focuses on use of ethanol which has no toxic effect and the combination would also give a cost effective approach. The results obtained in the present work suggest that hydro-alcoholic extraction can be applied for obtaining better yields.

The antioxidant activity of MOLE and PPE has been studied at different concentrations ranging from 100 to 900 ppm. Antioxidants tend to donate hydrogen and reduce unstable DPPH to diphenyl picrylhydrazine (Singh and Immanuel 2014). After reduction, the colour changes from deep violet to light yellow and absorbance at 517 nm is expected to decrease. This change in colour indicates the reduction of DPPH based on reaction with antioxidants present in the MOLE and PPE. The changes in antioxidant activity at different concentrations are represented in Table 1. The reported values were confirmed to be reproducible with multiple runs which also confirmed the validity of the data presented in the table. Higher concentrations of 900 ppm showed effective antioxidant activity of 95.1% for MOLE and 92.5% for PPE whereas antioxidant activity was less at lower concentrations of 300 ppm for both MOLE (91.2%) and PPE (88.3%). At the minimum concentrations used in the study as 100 ppm, the antioxidant activity was also much lower at 56.7% for the MOLE and 49.8% for the PPE, clearly confirming the relationship between the concentration of the antioxidants and the activity. Anwar et al. (2005) reported that moringa leaves are very rich in major phytochemicals such as ascorbic acid and flavonoids which are responsible for high antioxidant activity. Similar results were also reported by Makkar and Becker (1996) for the Moringa leaves demonstrated as very good natural antioxidants. Singh and Immanuel (2014) also reported that Pomegranate peels have greater content of polyphenols and flavonoids which exhibit higher antioxidant activity of 92.7%.

**Table 1** Effect of MOLE and PPE concentration on antioxidant activity

| Name of sample | Antioxidant activity (%) |
|---|---|
| MOLE 100 ppm | 56.7 |
| MOLE 150 ppm | 61.3 |
| MOLE 200 ppm | 67.3 |
| MOLE 300 ppm | 91.2 |
| MOLE 600 ppm | 93.4 |
| MOLE 900 ppm | 95.1 |
| PPE 100 ppm | 49.8 |
| PPE 150 ppm | 54.8 |
| PPE 200 ppm | 66.1 |
| PPE 300 ppm | 88.3 |
| PPE 600 ppm | 90.2 |
| PPE 900 ppm | 92.5 |
| BHT 100 ppm | 93.4 |
| BHA 100 ppm | 93.6 |

*MOLE Moringa oleifera* leaves extract; *PPE* Pomegranate peel extract; *BHT* butylated hydroxytoluene; *BHA* butylated hydroxyanisole

It is useful to also compare the antioxidant activity of the used natural extracts with the chemical antioxidants. In the present work, similar antioxidant activity was demonstrated by natural antioxidants (marginally higher for the MOLE) to that shown by the chemical antioxidants, clearly establishing the significance. Das et al. (2012) also reported that the antioxidant activity of MOL extract was found to be comparable to BHT. Zaki et al. (2015) reported that the use of pomegranate peel as natural antioxidant resulted in antioxidant activity of 80.21% that was lower compared to synthetic antioxidant (BHA) activity at 97.5%. Li et al. (2006) reported that pomegranate peel has more potential as natural antioxidants with high antioxidant activity mainly due to the presence of phenolics, flavonoids, and ascorbic acid. The acetone extract of M. oleifera leaves has also been demonstrated as fast and effective scavengers and its antioxidant activity of 95.27% with MOLE was marginally lower compared with BHT (98.47%) at a concentration of 1.0 mg/mL (Moyo 2012). Anwar et al. (2006) reported that moringa leaves showed slightly lower antioxidant activity of 93.5% compared to BHT (94.5%) but it was better compared to BHA (86%). Thus, it is clearly demonstrated that MOLE and PPE show very good antioxidant activity similar to that

demonstrated by the synthetic chemicals, which can prevent the oxidation of the oils and hence there is commercial importance for the obtained data. The presented comparison also confirmed that the suitability of the natural antioxidants and observed antioxidant activity trends is specific to the system used and hence the novelty of the current work dealing with edible oil blends is established.

## Antimicrobial activity

The antimicrobial activity of MOLE and PPE against both Gram-positive and Gram-negative strains was evaluated using different concentrations of extracts as 300 ppm, 600 ppm and 900 ppm. The obtained results for the inhibition zone are represented in Table 2. The size of inhibition zone states the ability of the extract to inhibit the growth of microbes. It was clearly established in the current work that MOLE and PPE components showed better antimicrobial effectiveness against both Gram-positive and Gram-negative strains. Analysis of the presented results established that high concentrations of MOLE and PPE exhibit high antimicrobial activity. Inhibition of 15.6 mm was observed against *Bacillus cereus* whereas 19.2 mm was the inhibition zone obtained against *Staphylococcus aureus* at concentration of 900 ppm of MOLE. Tiwari et al. (2009) also reported higher antimicrobial effectiveness against Gram-positive bacteria at higher concentration compared to lower concentration. The attributed mechanism was the irreversible modification of the cells and resulting death of microbes. Nadeem et al. (2013) reported that the effective penetration within the cell causes denaturation of protein and enzyme and finally death of the microorganism. The concentration of extracts was showed to play an important role in inhibition of microbes as also demonstrated in the current work. Lower concentrations of MOLE and PPE showed antimicrobial property but inhibition zone was small. At higher concentrations of 900 ppm, the inhibition zone increased significantly. Malviya (2014) reported that pomegranate peel exhibit strong antimicrobial property due to the presence of antioxidants and inhibit growth of staphylococcus aureus to a zone of 22.9 mm on the agar plate. Voravuthikunchai et al. (2005) also reported inhibitory effects of active compounds from pomegranate peel extracted with ethanol for the growth of Escherichia coli with minimum inhibitory concentration of 0.05 mg/ml. Khan and Hanee (2011) reported that ethanolic extracts of Pomegranate peel showed good antibacterial activity due to presence of antimicrobial compounds and zone of inhibition was 22 mm against *Pseudomonas aeruginosa* and 22.5 mm against *Escherichia coli*, both greater than the control samples. Pal et al. (1995) reported that antimicrobial screening of the leaf extract of Moringa leaves showed antimicrobial properties against Gram positive and negative strains with zone of inhibition as 15 mm for *Bacillus cereus*, 13 mm for *Staphylococcus aureus* and 19 mm for *Escherichia coli*. Devendra et al. (2011) reported that moringa leaves showed very good antimicrobial property against *Escherichia coli* with inhibition zone of 8.8 mm, against *Pseudomonas aeruginosa* with inhibition zone of 9.5 mm, and against *Staphylococcus aureus* with inhibition zone of 6.2 mm. Presence of various antimicrobial agents in both MOLE and PPE are responsible for high antimicrobial activities of the extracts. Based on the obtained results in the present work, it can be stated that concentration of 900 ppm is the best concentration to inhibit growth of microbes in the blends of oils to a maximum extent. It is important to note that though similar trends are obtained, the exact zone of inhibition and the best concentrations are dependent on the specific system. This analysis clearly established that detailed investigation as presented in the current work is important.

## Determination of oxidative stability by Rancimat analysis

The oxidative stability of blends was studied using the rancimat analysis. The induction time (IT) is time required for suitable oxidation of the blends such that the oil turns rancid. The oxidative study of blends containing natural antioxidants and controls was carried out and the obtained results for the study are shown in Table 3. The reported induction time (IT as represented in Table 3) is time required for the oil to be completely oxidized at elevated temperature of 120 °C. Induction period obtained for the control was compared

**Table 2** Effect of different concentrations of MOLE and PPE on Antimicrobial activity

| Organism | 300 ppm (MOLE) | 600 ppm (MOLE) | 900 ppm (MOLE) | 300 ppm (PPE) | 600 ppm (PPE) | 900 ppm (PPE) |
|---|---|---|---|---|---|---|
| Gram positive | | | | | | |
| *Bacillus cereus* | 7.3 mm | 9.2 mm | 15.6 mm | 8.4 mm | 12.3 mm | 16.9 mm |
| *Staphylococcus aureus* | 9.3 mm | 12.2 mm | 19.2 mm | 10.2 mm | 14.5 mm | 21.4 mm |
| Gram negative | | | | | | |
| *Escherichia coli* | 6.3 mm | 9.4 mm | 13.2 mm | 6.8 mm | 10.5 mm | 14.5 mm |
| *Pseudomonas aeruginosa* | 10.2 mm | 15.3 mm | 22.2 mm | 10.3 mm | 16.1 mm | 22.5 mm |

*MOLE Moringa oleifera* leaves extract; *PPE* Pomegranate peel extract

**Table 3** Results of rancimat study for stability in terms of the induction time of blends

| Name of Blend (80: 20) | IT CON | IT (MOLE) 300 ppm | IT (MOLE) 600 ppm | IT (MOLE) 900 ppm | IT (PPE) 300 ppm | IT (PPE) 600 ppm | IT (PPE) 900 ppm | IT (BHT) 100 ppm | IT (BHA) 100 ppm |
|---|---|---|---|---|---|---|---|---|---|
| Rice bran oil + MCT | 8.52 | 9.23 | 10.21 | 11.42 | 9.46 | 10.43 | 11.56 | 12.05 | 11.59 |
| Coconut oil + MCT | 30.1 | 32.4 | 34.5 | 36.42 | 32.57 | 34.32 | 36.48 | 37.01 | 36.53 |
| Groundnut oil + MCT | 3.24 | 5.34 | 7.31 | 9.45 | 4.54 | 7.01 | 9.23 | 9.34 | 9.56 |
| Sunflower oil + MCT | 1.99 | 3.21 | 5.23 | 6.57 | 3.22 | 5.32 | 6.59 | 7.07 | 7.03 |
| Soybean oil + MCT | 3.40 | 6.37 | 7.45 | 9.51 | 6.41 | 7.11 | 9.46 | 9.58 | 9.48 |
| Palm oil + MCT | 10.1 | 13.3 | 15.47 | 17.54 | 13.51 | 15.52 | 17.23 | 18.21 | 18.31 |
| Moringa oil + MCT | 9.75 h | 13.54 | 15.58 | 18.02 | 13.23 | 15.45 | 17.56 | 18.45 | 18.52 |
| Olive oil + MCT | 10.12 | 14.31 | 16.23 | 18.54 | 14.10 | 17.23 | 18.59 | 19.03 | 18.57 |
| Fish oil + MCT | 0.35 h | 0.55 | 1.05 | 1.45 | 0.48 | 1.21 | 1.54 | 1.59 | 1.54 |
| Flaxseed oil + MCT | 0.55 | 1.20 | 1.45 | 3.45 | 1.38 | 1.58 | 3.56 | 4.05 | 3.23 |

*IT* Induction time; *MCT* Medium chain triglycerides; *CON* Control run without any anti-oxidant; *MOLE Moringa oleifera* leaves extract; *PPE* Pomegranate peel extract; *BHT* butylated hydroxytoluene; *BHA* butylated hydroxyanisole

with samples containing MOLE and PPE. Higher induction time of 36.48 h for PPE and 36.42 h for MOLE compared to control of 30.1 h in all blends was demonstrated for the blend of Coconut oil and MCT. The obtained trends for the higher induction times at higher concentrations of MOLE or the PPE augur well with the DPPH activity results confirming the correspondence between the DPPH activity and the induction time. The better antioxidant activity confirmed from the DPPH results confirm that it will take more time for complete oxidation as reflected by the higher induction times. The obtained induction time with the natural antioxidants was only marginally lower compared with synthetic antioxidants with the actual time as 36.53 h for BHA and 37.01 h for BHQ. It is important to note that the trends were confirmed based on the repeated experiments and data is not within the experimental limits. Thus, MOLE and PPE showed induction time similar to chemical antioxidants and hence can be used as sustainable alternative to harmful chemical antioxidants to be used to prevent oxidation of blends of oils over long-term storage.

The data reported in Table 3 confirmed that blending of LCT and MCT with incorporation of MOLE and PPE resulted in marked increase in induction periods for all the blends indicating enhanced oxidative stability in the presence of natural antioxidants. However the actual induction period and the extent of increase were different for each combination of the LCT and MCT. For example, the induction period was highest for coconut oil followed by olive oil and moringa oil blended with MCT, MOLE and PPE. Maximum induction period for coconut oil can be attributed to the presence of saturated fatty acids, which are relatively more stable. In the case of flax and fish oils, there are a lot of n-3 fatty acids, which are very unstable due to double bonds yielding lower induction times. It was also demonstrated that the extent of increase for the best concentration of 900 ppm of MOLE and PPE was the maximum attributed to higher antioxidant activity at higher concentrations of MOLE and PPE. Similar results can be seen in the literature though usually for a fixed set of oil/natural extract combination. For example, Chatha et al. (2011) reported oxidation of canola oil was delayed using natural antioxidant from wheat bran extract. Chandran et al. (2017) reported higher antioxidant activity of natural antioxidants increasing shelf life of the vegetable oils and were thus recommended as replacement for the carcinogenic chemical antioxidants. Abd-Allah et al. (2018) also reported effectiveness of pomegranate peel as antioxidant to prevent oxidation of sunflower oil (SFO) and soybean oil (SBO). In their work, Rancimat study revealed that induction period of sunflower oil and soybean oil applied as control was found to be 1.99 h and 3.40 h respectively whereas induction time increased to 9.59 h and 9.51 h respectively in the presence of antioxidants. Nascimento et al. (2014) studied rancimat analysis of soybean oil incorporated with moringa leaves extract as an antioxidant and reported that induction time increased from 10.56 h to 12.97 h due to addition of natural antioxidant. El-Shourbagy and El-Zahar (2014) studied the effect of natural antioxidants extracted from pomegranate peel on oxidative stability of ghee and reported that induction time was the highest as 24.3 h for ghee enriched with BHA whereas ghee enriched with Pomegranate peel showed induction time of 17.4 h and the lowest induction time was seen for control with actual value as 15.3 h. Nadeem et al. (2015) investigated oxidative stabilization of blends of different vegetable oils at ambient temperature using different concentrations of ethanolic extracts of moringa leaves. Induction period of control sample, oil blends containing Moringa leaves and oil blends containing TBHQ was 3.46, 7.95 and 8.57 h respectively. Thus, it can be stated from the analysis of samples with rancimat that MOLE and PPE were potential in preventing oxidation

**Table 4** Results for peroxide value at ambient temperature of storage

| Name of Blend (80: 20) | PV CON0 days | PV CON 30 days | PV CON 60 days | PV CON 90 days | PV MO 30 days | PV MO 60 days | PV MO 90 days | PV PP 30 days | PV PP 60 days | PV PP 90 days | PV BHT 30 days | PV BHT 60 days | PV BHT 90 days | PV BHA 30 days | PV BHA 60 days | PV BHA 90 days |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rice bran oil + MCT | 0.26 | 2.1 | 5.1 | 6.1 | 1.3 | 2.5 | 3.4 | 1.4 | 2.1 | 3.5 | 1.2 | 2.1 | 3.2 | 1.2 | 2.2 | 3.3 |
| Coconut oil + MCT | 0.49 | 1.7 | 4.4 | 5.6 | 1.0 | 2.1 | 2.8 | 1.2 | 2.3 | 2.7 | 1.3 | 2.2 | 2.6 | 1.2 | 2.1 | 2.5 |
| Groundnut oil + MCT | 0.54 | 2.4 | 5.7 | 8.1 | 1.5 | 2.6 | 3.8 | 1.3 | 2.3 | 3.6 | 1.2 | 2.4 | 3.3 | 1.4 | 2.7 | 3.5 |
| Sunflower oil + MCT | 0.67 | 4.1 | 6.5 | 9.1 | 2.3 | 3.5 | 4.6 | 2.7 | 3.2 | 4.7 | 2.5 | 3.5 | 4.2 | 2.4 | 3.3 | 4.3 |
| Soybean oil + MCT | 0.89 | 2.0 | 5.7 | 7.3 | 1.3 | 2.7 | 4.3 | 1.4 | 2.5 | 4.2 | 1.2 | 2.5 | 4.1 | 1.3 | 2.3 | 4.2 |
| Palm oil + MCT | 0.51 | 1.9 | 4.3 | 5.5 | 1.2 | 2.3 | 4.3 | 1.3 | 2.4 | 4.5 | 1.4 | 2.5 | 4.6 | 1.3 | 2.4 | 4.3 |
| Moringa oil + MCT | 0.65 | 1.8 | 4.1 | 5.7 | 1 | 2.7 | 4.3 | 1.1 | 2.5 | 4.2 | 1.2 | 2.4 | 4.1 | 1.3 | 2.3 | 4.3 |
| Olive oil + MCT | 0.52 | 1.6 | 3.5 | 5.1 | 1.0 | 2.1 | 2.9 | 1.2 | 2 | 2.7 | 1.3 | 2.2 | 2.9 | 1.4 | 2.3 | 2.7 |
| Fish oil + MCT | 3.4 | 8 | 14 | 20 | 3.4 | 7.2 | 10.4 | 3.3 | 7.3 | 10 | 3.5 | 7.8 | 10.4 | 3.6 | 7.7 | 11 |
| Flaxseed oil + MCT | 2.21 | 6.3 | 10.3 | 13.2 | 2.5 | 3.8 | 7.1 | 2.4 | 3.6 | 7.0 | 2.4 | 3.5 | 7.4 | 2.3 | 3.7 | 7.5 |

*PV* Peroxide value; *MCT* Medium chain triglycerides; *CON* Control run without any anti-oxidant; *MO Moringa oleifera* leaves extract; *PP* Pomegranate peel extract; *BHT* butylated hydroxy-toluene; *BHA* butylated hydroxyanisole

**Table 5** Results for peroxide value at elevated temperature (60 °C) of storage

| Name of Blend (80: 20) | PV CON 0 days | PV CON 5 days | PV CON 10 days | PV CON 15 days | PV MO 5 days | PV MO 10 days | PV MO 15 days | PV PP 5 days | PV PP 10 days | PV PP 15 days | PV BHT 5 days | PV BHT 10 days | PV BHT 15 days | PV BHA 5 days | PV BHA 10 days | PV BHA 15 days |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rice bran oil + MCT | 0.26 | 5.2 | 10.2 | 17.6 | 3.2 | 5.6 | 8.9 | 3.5 | 5.8 | 9.1 | 3.3 | 5.4 | 8.8 | 3.2 | 5.3 | 8.9 |
| Coconut oil + MCT | 0.49 | 4.6 | 7.3 | 13.1 | 2.7 | 5.4 | 8.2 | 2.9 | 5.6 | 8.7 | 2.4 | 5.2 | 8.2 | 2.5 | 5.3 | 8.5 |
| Groundnut oil + MCT | 0.54 | 8.2 | 16.3 | 38.1 | 4.3 | 8.4 | 15.8 | 4.7 | 8.6 | 16.2 | 4.5 | 8.3 | 16.1 | 4.6 | 8.1 | 15.7 |
| Sunflower oil + MCT | 0.67 | 8.1 | 24.1 | 50.3 | 3.8 | 6.8 | 17.8 | 3.4 | 7.1 | 18.9 | 4.2 | 8.2 | 20.4 | 4.5 | 8.9 | 20.9 |
| Soybean oil + MCT | 0.89 | 6.4 | 18.7 | 35.2 | 3.1 | 9.4 | 14.5 | 2.8 | 7.9 | 13.8 | 3.2 | 7.1 | 15.1 | 3.1 | 6.8 | 15.5 |
| Palm oil + MCT | 0.51 | 5.5 | 10.4 | 17.2 | 2.9 | 5.8 | 8.6 | 2.8 | 5.4 | 8.7 | 3.0 | 5.6 | 8.8 | 3.1 | 5.7 | 8.8 |
| Moringa oil + MCT | 0.65 | 5.3 | 9.4 | 17.6 | 3.1 | 5.4 | 8.8 | 3.4 | 5.8 | 8.6 | 3.5 | 5.9 | 8.9 | 3.6 | 6.0 | 9.0 |
| Olive oil + MCT | 0.52 | 4.9 | 8.4 | 16.2 | 2.4 | 5.2 | 8.4 | 2.5 | 5.1 | 8.3 | 2.2 | 5.0 | 8.1 | 2.3 | 5.1 | 8.6 |
| Fish oil + MCT | 3.4 | 13.3 | 26.7 | 54.1 | 7.3 | 13.9 | 23.5 | 8.3 | 14.3 | 24.8 | 8.5 | 15.2 | 24.7 | 8.3 | 14.8 | 25.4 |
| Flaxseed oil + MCT | 2.21 | 10.3 | 20.4 | 34.5 | 6.8 | 12.3 | 22.8 | 8.1 | 13.7 | 24.3 | 8.1 | 14.8 | 23.8 | 8.1 | 14.3 | 24.3 |

*PV* Peroxide value; *MCT* Medium chain triglycerides; *CON* Control run without any anti-oxidant; *MO Moringa oleifera* leaves extract; *PP* Pomegranate peel extract; *BHT* butylated hydroxy-toluene; *BHA* butylated hydroxyanisole

of the blends and induction time increased indicating better shelf life for the products. The obtained results and the comparison with the literature clearly established the utility of the current work dealing with different combination as it has enabled to conclusively understand the dependency of changes in the induction time as a function of the type of vegetable oil or its blend as well as the natural extract applied as the antioxidant.

### Effect of time and temperature on peroxide value established using Schaal oven test

Schaal oven test was performed to check the oxidative stability of the blends in the presence of natural antioxidants (MOLE and PPE) at two different temperatures as 30 °C (ambient conditions) for 3 months and 60 °C (elevated) for 15 days. The peroxide value test was used to quantify the oxidative stability of the blends. The obtained data represented in Table 4 (for ambient conditions) and Table 5 (at elevated temperature of 60 °C) clearly demonstrated the increase in peroxide value (PV) for the blends of oils at normal storage (3 months period at ambient temperature) and accelerated storage (15 days at 60 °C) respectively. It is seen that the peroxide value increased gradually during the storage period of 3 months at ambient temperature. On the other hand, higher increase in PV value was obtained at elevated temperatures and also for the samples without control compared to the samples containing natural antioxidants. The control showed highest value of PV of 54 meq/kg after storage at accelerated temperature conditions for fish oil and MCT blend whereas with MOLE and PPE, the value was reduced to 23.4 meq/kg and 24.8 meq/kg respectively. Slow rise in PV of all samples with MOLE and PPE compared to control indicates good antioxidant activity of the extracts and hence it can be concluded that MOLE and PPE are effective in preventing the oxidation of the blends. The obtained results for the lower changes in the PV value also correlate well with the DPPH activity results confirming the correspondence. The better antioxidant activity confirmed from the DPPH results confirm that there will be less oxidation and hence lower changes in the PV value. The activity for preventing oxidation was due to presence of high level of phenolic compounds in the extracts that definitely help in retarding oxidation. The measurements of the total polyphenol content revealed that the total polyphenol for the MOLE was 1419 mg of GAE equivalent/100 gm of sample whereas that for PPE was 1014 mg of GAE equivalent/100 gm of sample.

The increase in the peroxide value was the least for coconut oil and moringa oil whereas highest increase was seen for flaxseed and fish oil confirming lower oxidative stability observed similar to the induction time results discussed earlier. The composition of the oil indeed plays a role in deciding the stability of oils. It is important to note that for the commonly used edible oils, use of MOLE and PPE is demonstrating very good results confirming the commercial utility of the presented work. Similar results can be seen in the literature though with usage of fixed oils. For example, Nadeem et al. (2013) reported that MOLE showed strong antioxidant activity in terms of preventing butter oil from becoming rancid. Anwar et al. (2007) also reported that moringa oleifera leaves extract (concentration of 600 ppm) maintained stability of sunflower oil (SFO). Turgut et al. (2017) investigated efficiency of pomegranate peel in retarding lipid oxidation and reported that pomegranate extract exhibits high antioxidant effect and yields stability of more than 6 months during frozen storage of meatballs. Bopitiya and Madhujith (2014) also reported that pomegranate peel extract exhibited strong antioxidant activity, even superior, compared to that of chemical antioxidants as peroxide value increased very slowly from 0.78 meq/kg to 2.3 meq/kg during 3 consecutive frying cycles. Abd-Allah et al. (2018) also reported that peroxide value of sunflower oil at elevated temperature of 65 °C was 58.18 meq/ kg and with the addition of chemical antioxidant BHT, it reduced to 25.82 meq/kg whereas with pomegranate peel extract, stronger reduction to 16.21 meq/kg of sunflower oil was obtained. Bashir et al. (2016) also reported that peroxide value of sunflower oil at ambient temperature increased from 1.97 meq/kg to 7.13 meq/ kg for pomegranate peel extract whereas the final value was 17.64 meq/kg for control after similar storage time of 30 days. The obtained results in the present work and comparison with literature established the efficacy of natural antioxidants and importance of the work dealing with the different blends of MCT with moringa oil, olive oil, rice bran oil, and coconut oil. It is clearly seen that the level of stability obtained using MOLE and PPE was dependent on the specific combination and hence this also suggests that different concentrations of natural antioxidants can be added into the blends for similar results of stability.

## Conclusions

The results of the present study clearly established that moringa leaves extract and pomegranate peel extract at loading of 900 ppm can be effectively used as natural antioxidants. The obtained effects are comparable or at times better than that demonstrated by the chemical antioxidants such as BHA and BHT. Different studies such as antimicrobial analysis, the induction time measurements and the peroxide value analysis clearly confirmed the beneficial role of using MOLE and PPE. Overall, it can stated that the carcinogenic

chemical antioxidants can be effectively replaced by the natural antioxidants in the applications as edible oils, and also during nutraceutical and food applications. Natural extracts clearly retarded the rate of oxidations and the presented results on effect of temperature are very important for commercial applications.

**Acknowledgements** SBM is grateful to Dr. Babasaheb Ambedkar National Research and Training Institute (BARTI) for financial support for the Ph.D. fellowship. JSW and PRG acknowledge the efforts of Mr. Harsh Jadhav for the assistance in the analysis related to DPPH activity and total polyphenol content.

## Declarations

**Conflict of interest** Authors have no conflict of interest to declare. Authors also declare that there is no funding that could have influenced the studies reported in the work.

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