Protection of Apricot Biodiesel from Thermal Degradation by using Natural Antioxidants of *Fagopyrum tataricum* (L.) Gaertn.

*FAIZAN ULLAH, ARSHAD HUSSAIN WAZIR*, ADNAN KHATTAK, SHAHAN ZEB KHAN & ISHTIAQ HUSSAIN

**ABSTRACT**

The present study aims to improve the oxidation stability of wild apricot kernel oil biodiesel (WAKOB) by using natural antioxidants of Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.). Biodiesel was synthesized at different catalyst (NaOH) concentrations, reaction temperatures, reaction time intervals and methanol-to-oil molar ratios. Thermal oxidative stability measurements were carried out according to EN14112 using a Rancimat instrument. Our results showed a high yield of biodiesel (97±1.092) at 65°C in the presence of 1% NaOH (%w/w oil) and methanol/oil molar ratio of 9:1 and for the time duration of 60 min. Proton nuclear magnetic resonance (1H NMR) confirmed the conversion percentage of kernel oil into biodiesel, which was further evidenced by Fourier transform infrared spectroscopy (FT-IR) and refractometer analyses. Methanolic fraction of Tartary buckwheat leaves (MTBWLF) was standardized to contain the highest amount of phenolics (209 mg gallic acid/100 g). In this study, the mixture of synthetic antioxidant butylated hydroxyl toluene (BHT) (0.25%) and methanolic extract of Tartary buckwheat leaves (0.5%) ensured high oxidation stability of biodiesel samples, leading to stabilizing factor of 4.86.

**Keywords:** Biodiesel; FT-IR spectroscopy; ¹H NMR spectroscopy; natural antioxidant

**ABSTRAK**

Kajian ini bertujuan untuk memperbaiki kestabilan pengoksidaan minyak kernel aprikot liar biodiesel (WAKOB) dengan menggunakan antioksidan semula jadi semula jadi dari gandum buck Tartary (*Fagopyrum tataricum* (L.) Gaertn.). Biodiesel telah disintesis pada kepekatan pemangkin berbeza (NaOH), tindak balas suhu, tindak balas reaksi selang masa dan nisbah molar metanol kepada minyak. Pengukuran kestabilan termal oksidatif telah dijalankan mengikut EN14112 dengan menggunakan instrumen Rancimat. Keputusan kami menunjukkan hasil biodiesel yang tinggi (97±1.092) pada 65°C kehadiran 1% NaOH (%w/w minyak) dan metanol/ nisbah molar minyak 9:1 dan untuk tempoh masa 60 min. Nuklear proton resonans magnetik (1H NMR) mengesahkan peratusan penukaran minyak isirong kepada biodiesel, dan dibuktikan seterusnya oleh spektroskopi transformasi Fourier Inframerah (FT-IR) dan analisis refraktometer. Pecahan metanolik daun gandum buck Tartary (MTBWLF) telah dipiawaikan untuk mengandungi jumlah tertinggi fenolik (209 mg asid galik/100 g). Dalam kajian ini, campuran sintetik antioksidan butilated toluen hidroksil (BHT) (0.25%) dan ekstrak metanolik daun gandum buck Tartary (0.5%) memastikan kestabilan pengoksidaan sampel biodiesel yang tinggi yang membawa kepada penstabilan faktor 4.86.

**Kata kunci:** Antioksidan semula jadi; biodiesel; spektroskopi FT-IR; spektroskopi ¹H NMR

**INTRODUCTION**

Rapid population growth and industrial development has resulted an increase in energy consumption with a concomitant increase in the price of crude petroleum. Moreover, greenhouse gas emission by the usage of fossil fuels is also becoming a greater concern (Rizwanul et al. 2014). Therefore, due to environmental concern and non-renewable nature, organizations are avoiding fossil fuel and have boosted research on alternative renewable resources of energy. Biodiesel is an alternative, non-toxic and environment-friendly fuel. Biodiesel has the ability to potentially decrease the exhaust emissions of carbon dioxide, carbon monoxide, sulfur and nitrogen oxides, particulate matter, volatile organic compounds and unignited hydrocarbons as compared to the fossil fuels (Mofijur 2012). In conclusion, biodiesel is biodegradable and hence environment friendly that make it an ideal for use as an alternative to fossil fuel. Chemically biodiesel is a mixture of mono alkyl esters of long chain fatty acids produced from vegetable oil or animal fat through a process called transesterification (Buyukkaya et al. 2013). Biodiesel production is a multistep process that is influenced by various variables such as catalyst concentration, temperature, methanol/oil molar ratio and reaction time (Mofijur et al. 2012; Ullah et al. 2013). A great majority of edible and non-edible vegetable oil resources are exploited for biodiesel production. In Pakistan wild apricot (*Prunus armeniaca* L.) grows at a
high altitude of 1000 - 2700 m above sea level wherein, their kernels have the potential to yield 48% oil (Ullah et al. 2009). The fruit yield of wild apricot is round about 24,694 tons per year in Gilgit-Baltistan of Pakistan (Anon 2010). Its oil is non-edible due to higher content of amygdalin with bitter taste. Therefore, its use in biodiesel production is highly recommended. Just like vegetable oil or fat, biodiesel also undergoes degradation during storage, mainly influenced by factors such as oxygen, temperature and light. The degradation products of biodiesel such as formation of organic acids, gums and sediments cause engine problems (Desouza et al., 2013; Nazesh 2015; System Lab Services 1997). Therefore, it is necessary to check oxidation stability of biodiesel. The method commonly used for determination of the oxidation stability of biodiesel is the so called Rancimat method. This method is well correlated with other biodiesel parameters and is therefore a suitable instrument to evaluate oxidation stability (Gregory 2014; Schober et al. 2004). The standard oxidation stability is 6 h at 110°C (Chaitongdee et al. 2010). Plant oils contain natural antioxidants that are degraded during the process of biodiesel production, which result biodiesel susceptible to oxidation (Desouza et al. 2013). Addition of antioxidants in biodiesel has the ability to delay the rate of lipid oxidation in order to inhibit the free radical formation (Ling et al. 2006). Synthetic antioxidant like butylated hydroxy toluene (BHT) is very effective in the protection of polyunsaturated fatty acids. However, the use of these synthetic antioxidants is discouraged because of their toxicity and carcinogenicity (Wazir 2015). Natural antioxidants reduce the use of synthetic antioxidants in biodiesel efficiency when practiced in combination. Natural antioxidants are of common occurrence in many fruits and vegetables that have attracted a great deal of public and scientific attention because of their anticarcinogenic potential. The methanolic extract of Jatropha curcas roots and ethyl ether extract of oregano was highly efficient in protecting biodiesel from thermal degradation. Tartary buckwheat (Polygonaceae) has higher antioxidant activity and is a good source of polyphenol compounds (i.e. Benzene rings with one or more hydroxyl groups such as flavonoids and phenolic acids) (Guo et al. 2011; Naczek et al. 2015). The polyphenols exhibit more phenolic hydroxyl groups which are indicative of higher antioxidant activity (Diwani et al. 2009). In Pakistan, buckwheat is cultivated on 57% of total area in the Gilgit-Baltistan. Buckwheat has a short life cycle and therefore it can be grown on marginal lands (Anon 2010). Utilization of leaves of Tartary buckwheat as source of natural antioxidants for biodiesel can be a feasible option and will not compete with food products. It is hypothesized that natural antioxidants of Tartary buckwheat would be an effective alternative to synthetic antioxidants for improving oxidative stability of wild apricot biodiesel by scavenging the generation of reactive oxygen species. The major objective of the present study was to optimize protocols for biodiesel production from wild apricot kernel oil and to formulate the stabilization process for the storage lifetime time of the product with natural antioxidant.

MATERIALS AND METHODS
The fully ripened wild apricot fruits (5 kg) were collected from trees growing under natural conditions in Gilgit-Baltistan region of Pakistan. The leaves of Tartary buckwheat were also collected in the month of July-August, 2013 from the District of Khaplu in Gilgit-Baltistan, Pakistan. The voucher plant specimen was deposited in the herbarium of University of Science and Technology, Bannu, KP, Pakistan.

BIO DIESEL SYNTHESIS
After breaking the stones of the fully ripened wild apricot fruits with mechanical decorticator were used first to remove an edible portion and then kernels were separated from the mass of the stones/pits with the specific gravity separation method. The moisture contents in a seed were removed by incubating the kernels in an oven for overnight at 30°C. The oil contents in kernels were extracted through a Soxhlet extraction method using petroleum ether as solvent. Furthermore, for biodiesel production, the crude oil was subjected to base catalyzed transesterification reaction. The pellets of sodium hydroxide at 1% and 0.5% (w/w oil) were dissolved in methanol, mixed with kernel oil and stirred on a magnetic stirrer. Two molar ratios of methanol to oil (6:1 and 9:1) were used at various temperatures (40, 50, 60, 65 and 70°C) (Ullah et al. 2013). After the transesterification reaction the mixture was placed for overnight to isolate into biodiesel and glycerol phases. In order to remove excessive methanol, catalyst and soap produced during the reaction, biodiesel phase was washed with warm distilled water up to 40°C and subsequently dried by using rotary film evaporator (RFE) at 120 rpm for 1 h at 35°C. The yield of biodiesel was determined on % w/w conversion of apricot kernel oil to biodiesel.

CHARACTERIZATION
The proton nuclear magnetic resonance spectrometer (HNMR) analysis of both the samples of crude apricot kernel oil and biodiesel were performed using Avan CE 300 MHz spectrometer (Bruker Avance III 300 MHz/54 mm NMR spectrometer, Billerica, MA, USA) equipped with 5 mm Broad Band Observe (BBO) probes at 7.05 T. After analysis, the transesterification yield was calculated with (1) as follows:

\[ C = 100 \times \frac{2 \text{AMe}}{3 \text{ACH}_2}, \]  

where \( C \) represents the percentage conversion of triglycerides to corresponding methyl esters; AMe shows the integer value of the methoxy protons of the methyl esters; and ACH2 present integration value of \( \alpha \)-methylene protons.
For FT-IR analysis, the samples were characterized with FT-IR (Bio-Rad Excalibur Model FTS3000MX, California, USA) in the range of 4000-400 cm⁻¹ and the resolution was 1 cm⁻¹ with 15 scans.

Similarly, the refractive indexes of pure wild apricot kernel oil and biodiesel samples were also determined by using an Abbe refractor meter (Atago Tokyo, Japan).

PREPARATION OF PLANT EXTRACTS

Extracts were prepared by simple maceration technique. Plant material was shade dried at room temperature, ground to powder and preserved in a sealed plastic bag at ambient temperature prior to analysis. A total of 60 g sample of each plant powder was soaked in 600 mL methanol with constant shaking for 72 h and filtered. The filtrate was concentrated using a rotary evaporator and finally evaporated to dryness in a water bath. The crude methanolic extract was then partitioned successively with n-hexane, ethyl acetate and n-butanol to give four fractions; n-hexane (HTBWL), ethyl acetate (EAHBWL), n-butanol (BBHBL) and methanol (MTBWL) (Mann et al. 2010).

DETERMINATION OF TOTAL PHENOLIC CONTENT OF EXTRACTS

Determination of total phenolics was based on the Folin-Ciocalteau method (Adom et al. 2002). An extract of 125 μL was mixed with distilled water (500 μL) where 125 μL of Folin-Ciocalteau reagent was subsequently added. The mixture was allowed undisturbed for a total of 6 min. The volume was marked up to 3 mL by the addition of 1.25 mL of 7% aqueous sodium carbonate and 1 mL distilled water. The absorbance was measured at 760 nm using a UV/Vis spectrophotometer (Hitachi’s U-5100 Tokyo Japan) after incubation for 90 min in the dark. The measurements were compared to a standard curve prepared by using various concentrations of gallic acid solution. The concentration of total phenolics was expressed as mg gallic acid equivalents/100 g sample.

THERMALLY INDUCED OXIDATION OF BIODIESEL SAMPLES

The 0.5% (v/v) of different fractions of Tartary buckwheat leaves (HTBWL, EAHBWL, BBHBL and MTBWL) or 0.25% BHT (synthetic antioxidant) or combination of 0.5% of various extracts and 0.125% of BHT were added singly and, respectively to WAKOB and tested for thermal oxidation stability using a Metrohm 743 Rancimat instrument (Metrohm AG, Herisau Switzerland). The Rancimat test is a specified standard method for oxidative stability testing of biodiesel according to EN14112 (European Committee for Standardization 2003) as described earlier (Domingos et al. 2007; Sarin et al. 2007). Biodiesel samples each of 3 g were analyzed at a heating block temperature of 110°C and at constant air flow of 10 L/h. The effectivenss of all the tested antioxidants was expressed by the stabilization factor (Adom et al. 2002) as given in (2).

\[
F = \frac{IP_x}{IP_0},
\]  

where IPx is the induction period in presence of the antioxidant; and IP0 is induction period in the absence of the additive.

RESULTS AND DISCUSSION

Most of the characteristic properties of the obtained biodiesel were the same such as an acid value of 1.7(mg KOH g/oil), free fatty acid content of 0.84% (as oleic acid), the saponification number of 185(mg of KOH/g of oil), iodine value of 103(g 1/100 of oil) and specific gravity of 0.91 (g/cm³), as mentioned earlier in our previous published work (Ullah et al. 2009). While during the biodiesel formation process, our current results showed that the increase in temperature from 40°C to 65°C enhanced the product yield of biodiesel as shown in (Table 1). A positive correlation was observed among the increase in temperature, methanol/oil molar ratio and yield of biodiesel. The maximum yield (97%) of biodiesel was obtained at 65°C with 1% catalyst concentration (w/w) and methanol/oil molar ratio of 9:1 (Tables 1 & 2). A decrease in the content of biodiesel was recorded as the temperature increase was ensured beyond 65°C. The increase in methanol/oil molar ratio (9:1) was ineffective to decrease the adverse effects of a higher temperature on biodiesel yield. Similar results for the impact of temperature, catalyst concentration and methanol/oil molar ratio of biodiesel yield in base catalyzed transesterification reaction were reported by Ullah et al. (2013) for mustard oil and Ahmad et al. (2010) for the sesame oil. The temperature, alcohol/oil molar ratio and catalyst concentration are considered crucial for optimizing protocol for biodiesel production in an alkali catalyzed transesterification. It was found that wild apricot kernel oil needs a greater methanol excess for methanolysis in order to obtain a maximum conversion. The increase in incubation time from 30 to 60 min has showed that yield of biodiesel increased up to a maximum of (97%). Further increase in incubation time up to 120 min was found no effect on the yield of biodiesel. However, when the reaction time was increased beyond 150 min, the yield of biodiesel got decreased gradually (Figure 1). The reaction time is an important variable that affects yield and quality of biodiesel. Gradual increase occurs in the yield of methyl esters with the increase of reaction time (Mathiyazhagan et al. 2011). The maximum triglyceride conversion into methyl esters was achieved in a time period of <90 min (Alamu et al. 2007). The decrease in methyl esters yield after 150 min of reaction initiation might be due to the loss of methyl esters and formation of soap. An extended reaction time resulted in the loss of methyl esters due to the reversible reaction of transesterification and formation of soap (Eevera et al. 2009).

As it is well known that 'NMR spectroscopy is utilized as a powerful tool for determining the conversion of vegetable oil into biodiesel (Gelhard et al. 1995).
Therefore, in this study the obtained product yield of biodiesel was also confirmed via $^1$H NMR that presented a 97.1% conversion of apricot kernel oil into biodiesel as shown in (Table 2).

Similarly, the FT-IR spectra regarding to functional groups and the bands corresponding to various vibrations in WAKOB is shown in Figure 2(a), 2(b) and is also presented in Table 3. Same carbonyl groups with some novel bands were detected in the spectra of oil and biodiesel. The presence of a novel band at 1168.4 cm$^{-1}$ corresponding to methyl group replaced glycerol in oil and indicated the conversion of oil into biodiesel. Some sort of variation was observed in stretching vibration of carbonyl (νC=O) from 1743 and 1741 cm$^{-1}$ in oil and biodiesel, respectively. Glycerol in oil was replaced by a methoxy group attached at νC=O. There was observed a shifting of some absorption peaks of the oil sample at 1158.8 and 1094.9 cm$^{-1}$ to 1168.0 and 1016 cm$^{-1}$, respectively, in biodiesel. The stretching bands of methyl and methylene were revealed at 3006.5 and 2922.6 cm$^{-1}$ respectively, while peak at 2922.6 cm$^{-1}$ showed the appearance of the aliphatic C-H bond. The appearance of an absorption peak at 721 cm$^{-1}$ indicated the CH$\_2$ rocking. The refractive index of WAKOB was observed lower than pure kernel oil as shown in Figure 3. The biodiesel sample (S1) with a maximum methyl esters content (prepared with a 1% catalyst concentration, reaction temperature of 65°C and methanol/oil molar ratio of 9:1) exhibited the lowest refractive index (1.450 and 40°C). These values indicated that heavier molecules got converted into a lighter one during transesterification process (Deshpande et al. 2012). These results are also in confirmatory with those of Domínguez et al. (1996), who have reported a refractive index of 1.45 in pure biodiesel possesses.
TABLE 2. Biodiesel yield of wild apricot kernel oil calculated through \(^1\)H NMR

<table>
<thead>
<tr>
<th>Catalyst concentration (NaOH % w/w)</th>
<th>Methanol/oil molar ratio</th>
<th>Reaction temperature °C</th>
<th>Yield of biodiesel (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9:1</td>
<td>65</td>
<td>97.1</td>
</tr>
<tr>
<td>1</td>
<td>6:1</td>
<td>70</td>
<td>90.68</td>
</tr>
<tr>
<td>0.5</td>
<td>9:1</td>
<td>65</td>
<td>90.46</td>
</tr>
<tr>
<td>0.5</td>
<td>6:1</td>
<td>70</td>
<td>83.13</td>
</tr>
</tbody>
</table>

FIGURE 2. FTIR spectra for (a) wild apricot kernel oil (b) wild apricot kernel oil biodiesel

TABLE 3. FTIR analysis of wild apricot kernel oil biodiesel

<table>
<thead>
<tr>
<th>S.No</th>
<th>Functional Groups</th>
<th>Peaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methoxy (methyl)</td>
<td>1168.4</td>
</tr>
<tr>
<td>2</td>
<td>O-C</td>
<td>1016</td>
</tr>
<tr>
<td>3</td>
<td>Ester carbonyl group (C=O)</td>
<td>1741</td>
</tr>
<tr>
<td>4</td>
<td>Methylene (stretching)</td>
<td>1365</td>
</tr>
<tr>
<td>5</td>
<td>Methylene (bending)</td>
<td>2922</td>
</tr>
<tr>
<td>6</td>
<td>Terminal methyl (stretching)</td>
<td>1435.2</td>
</tr>
<tr>
<td>7</td>
<td>Terminal methyl (bending)</td>
<td>1853.2</td>
</tr>
<tr>
<td>8</td>
<td>Methine (stretching)</td>
<td>3006.5</td>
</tr>
</tbody>
</table>

OXIDATION STABILITY VERIFICATION

The total phenolic compounds content was found higher in MTBWLF followed by BTBWLF, EATBWLF and HTBWLF (Figure 4). Different solvents have been used for the extraction of polyphenols from plant materials and their efficiency varies. This may be due to the differences in polarity of the solvents used. Previous studies have shown that methanol found highly efficient in the extraction of polyphenols from black currant and grape byproducts (Lapornik et al. 2005).

The oxidation of lipids takes place in three distinct phases: Initiation or induction, propagation and ending (Moretto et al. 1998). In the initial phase peroxide is
generated which increase in number with time and tends to stabilize after a certain period (Storch et al. 2008). Oxidation rates of fatty acid methyl esters depend upon many variables such as temperature, light exposure and radiation intensity (Carelli et al. 2005). The results of our present study showed that generally all the tested antioxidants both synthetic and natural had a positive impact on the oxidation stability of WAKOB. Synthetic antioxidant BHT (0.5%) proved highly effective in leading to an increased induction period (20.16 h) and stabilization

TABLE 4. Induction periods and corresponding stabilization factors of WAKOB samples with synthetic and natural antioxidants

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Induction period (IP)</th>
<th>Stabilization factor (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>3.37</td>
<td></td>
</tr>
<tr>
<td>BHT (0.5%)</td>
<td>20.16</td>
<td>5.98</td>
</tr>
<tr>
<td>BHT (0.25%)</td>
<td>13.12</td>
<td>3.89</td>
</tr>
<tr>
<td>BHT (0.125%)</td>
<td>7.12</td>
<td>2.11</td>
</tr>
<tr>
<td>HTBWLF (0.5%)</td>
<td>4.15</td>
<td>1.23</td>
</tr>
<tr>
<td>EATBWLF (0.5%)</td>
<td>4.43</td>
<td>1.31</td>
</tr>
<tr>
<td>BTBWLF (0.5%)</td>
<td>4.61</td>
<td>1.37</td>
</tr>
<tr>
<td>MTBWLF (0.5%)</td>
<td>5.50</td>
<td>1.63</td>
</tr>
<tr>
<td>HTBWLF (0.5%) + BHT (0.25%)</td>
<td>14.30</td>
<td>4.24</td>
</tr>
<tr>
<td>EATBWLF (0.5%) + BHT (0.25%)</td>
<td>14.12</td>
<td>4.19</td>
</tr>
<tr>
<td>BTBWLF (0.5%) + BHT (0.25%)</td>
<td>14.18</td>
<td>4.21</td>
</tr>
<tr>
<td>MTBWLF (0.5%) + BHT (0.25%)</td>
<td>16.38</td>
<td>4.86</td>
</tr>
<tr>
<td>HTBWLF (0.5%) + BHT (0.125%)</td>
<td>8.14</td>
<td>2.42</td>
</tr>
<tr>
<td>EATBWLF (0.5%) + BHT (0.125%)</td>
<td>8.23</td>
<td>2.44</td>
</tr>
<tr>
<td>BTBWLF (0.5%) + BHT (0.125%)</td>
<td>9.27</td>
<td>2.75</td>
</tr>
<tr>
<td>MTBWLF (0.5%) + BHT (0.125%)</td>
<td>10.55</td>
<td>3.13</td>
</tr>
</tbody>
</table>

FIGURE 3. Refractive index of kernel oil and its respective biodiesel

S1- Biodiesel sample prepared at 65°C, 1% catalyst (w/w of oil), 9:1 methanol to oil ratio
S2- Biodiesel sample prepared at 65°C, 1% catalyst (w/w of oil), 6:1 methanol to oil ratio
S3- Biodiesel sample prepared at 70°C, 1% catalyst (w/w of oil), 9:1 methanol to oil ratio
S4- Biodiesel sample prepared at 70°C, 0.5% catalyst (w/w of oil), 9:1 methanol to oil ratio

FIGURE 4. Total phenolics content in leaves extracts of *Fagopyrum tataricum*
factor of 5.98. In the leaf extracts of *Fagopyrum tataricum*, MTBWLF was more effective than HTBWLF, EATBWLF and BTBWLF leading to a stabilization factor of 1.63. However, none of the natural additives increased the induction period to longer than the standard oxidation stability of 6 h. The effectiveness of MTBWLF was further improved in the presence of 0.125% BHT as evidenced by an increased induction period (16.38 h) and a significantly higher stabilization factor of 4.36 (Table 4). The antioxidants improve oxidative stability by removing free radicals formed during the oxidation initiation stage. Fatty peroxo radicals are thereby stabilized and the chain reaction is stopped (Carelli et al., 2005). The alcoholic extracts of oregano and rosemary were highly effective in improving oxidation stability of soybean biodiesel (Galvan et al., 2013). The antioxidant formulation should be of economical and environment friendly. Therefore, we assume that the most successful antioxidant formulation should be of those composed of 0.25% BHT and 0.5% methanolic extract of Tartary buckwheat. Natural antioxidants reduced the use of synthetic antioxidants in biodiesel efficiency when practiced in combination.

CONCLUSION

The maximum product yield of biodiesel formation in this work was found to be 97% at optimum conditions of reaction temperature of about 65°C, reaction time duration of 60 min, 1% of catalyst (NaOH) concentration and methanol/oil molar ratio of 9:1. The results obtained by ¹H NMR, FT-IR and refractometer represents that they can be utilized as powerful tools for confirming the conversion of wild apricot kernel oil into biodiesel. The addition of methanolic leaf extracts (0.5%) rendered the oxidative stability of biodiesel samples and the effect was found to be more pronounce in the presence of 0.25% BHT. It is suggested that the sensitive natural antioxidants of *Fagopyrum tataricum* are more economical, sustainable and environment friendly alternatives to prevent the oxidation of wild apricot kernel oil biodiesel during the storage and transportation processes. All authors have declared they have no conflicts of interests.

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Faizan Ullah & Adnan Khattak Department of Botany University of Science and Technology Bannu Khyber Pukhtunkhwa Pakistan

Arshad Hussain Wazir* & Shahan Zeb Khan Department of Chemistry University of Science and Technology Bannu Khyber Pukhtunkhwa Pakistan

Ishtiaq Hussain Department of Agriculture Gilgit-Baltistan Pakistan

*Corresponding author; email: arshadwaziri@gmail.com

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