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# Hydro-treatment of wild bitter apricot kernel oil: a cheap and cost-effective alternative for reducing toxicity and enhancing quality

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Wild bitter apricot kernel oil (AKO) is an underutilized resource due to the presence of cyanogenic glycosides (specifically amygdalin), which can pose toxicity during its direct utilization. This study explores the potential of hydro-treatment to detoxify AKO while preserving its physicochemical and nutritional parameters. The results revealed that non-treated apricot kernel oil possesses a yellow appearance, 1.468 refractive index and 0.92 specific gravity, and treated apricot kernel oil exhibits a pale-yellow appearance, 1.468 refractive index and 0.93 specific gravity. Notably, quantitative analysis revealed that the total phenolic content was higher in non-treated AKO (27.28  $\mu\text{g}$  GAE per g) compared to treated AKO (13.92  $\mu\text{g}$  GAE per g). AKO exhibited a comparable composition of prominent (especially oleic and linoleic acids) fatty acids. Both variants showed good antioxidant properties. Importantly, amygdalin was undetectable in the hydro-treated AKO, confirming its safety for utilization. Hydro-treatment did not significantly affect the yield, refractive index, specific gravity, fatty acid profile, acid value, iodine value, and peroxide and saponification values, with all parameters complying with the prescribed range of FAO/WHO and PFA specifications for almond oil. In conclusion, hydro-treatment is an effective approach to detoxify AKO, ensuring its safety and quality for potential applications in the food industry.

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## Sustainability spotlight

The sustainable hydro-treatment was applied to wild bitter apricot kernel oil. It converts a not fully tapped resource into a safe and functional food input. It minimizes harmful amygdalin while retaining the most critical physicochemical and nutritional characteristics. This approach is an example of a reasonable and responsible application of natural resources. It goes beyond conforming to international safety standards to increasing the prospect of utilising wild bitter apricot kernel oil as a sustainable food source, thereby advancing environmental and nutritional security.

## 1 Introduction

Fruit waste, which accounts for approximately 25–30% of global fruit commodities, is a rich source of bioactive compounds and nutrients.<sup>1</sup> Fruit waste, *i.e.* skin, peel, pomace, shells, seeds and kernels, contains various phytochemicals (flavonoids, polyphenols, and carotenoids), dietary fibres, vitamins, minerals, and enzymes. These compounds exhibit various biological activities, including antibacterial, antiviral, anti-tumoral, anti-mutagenic and cardio-protective actions.<sup>2,3</sup>

Nowadays, the interest of researchers has been garnered in the utilisation of underutilised seeds. Apricot (*Prunus armeniaca* L.) is a deciduous stone fruit belonging to the genus *Prunus*, subfamily *Prunoideae*, family *Rosaceae*, and order *Rosales*. This species is extensively cultivated in temperate climates

worldwide, particularly in regions with suitable chill hours and moderate winters. In India, *Prunus armeniaca* L. is primarily cultivated in the Himalayan foothills, specifically in the states of Himachal Pradesh, Jammu and Kashmir, and Uttarakhand. Notably, the genus *Armeniaca* is also grown in mountainous regions, although it is considered a synonym of *Prunus*.<sup>4,5</sup>

Apricot kernels can be grouped into two main categories based on their taste and amygdalin content, *i.e.* sweet kernel (Nyarmo) and bitter kernel (Khante).<sup>6</sup> Phytochemically, *Prunus* species seeds, including apricot kernels (bitter variety), are characterised by their high amygdalin content, ranging from 0.5 to 5.0%. Apricot kernels exhibit considerable variation in amygdalin content, influenced by several factors, including kernel type (sweet or bitter), geographical origin, and growing conditions. Sweet apricot kernels contain relatively low levels of amygdalin (less than 5  $\text{mg kg}^{-1}$ ), with respect to bitter kernels, having very high concentrations (more than 200  $\text{mg/100 g}$ ).<sup>7</sup> This variability in amygdalin content is significant for the potential applications as well as the safety of apricot kernels. In

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addition, the seeds of multiple *Prunus* spp, such as *P. dulcis* var. amara (bitter almond), *P. armeniaca* (apricot), *P. avium* (cherry), *P. avium* var. sativa (sweet cherry), *P. persica* (peach), *P. domestica* (plum), *P. persica* var. nectarina (nectarine) and *Malus domestica* (apple) contain a high amount of amygdalin.<sup>8</sup> When the seed is ruptured, the amygdalin is enzymatically hydrolysed into glucose, benzaldehyde and hydrogen cyanide (HCN), which causes cyanide poisoning. The toxicological threshold of cyanide in humans is around 1.5 mg kg<sup>-1</sup>.<sup>9</sup>

Apricot kernel serves as a valuable source of high-quality oil and protein, along with essential minerals, vitamins and phenolic compounds.<sup>10</sup> Due to the high content of mono- and polyunsaturated fatty acids and natural antioxidants such as polyphenols and tocopherols, apricot kernel oil is used as edible oil in salad dressing, confectionery and gourmet oils. It is also used as an ingredient in medicinal supplements and pharmaceutical products due to its cardioprotective, antioxidant, and anti-inflammatory properties. Due to the similar fatty acid profile to almond oil, apricot kernel oil is also used as a substitute in food and cosmetic product formulations.<sup>11</sup>

Despite the high oil content, bitter apricot kernels remain underutilised, primarily due to the presence of toxic cyanogenic glycosides, among which amygdalin is the most prevalent. During oil extraction, a considerable amount of amygdalin is transferred from bitter apricot kernels to its oil. According to Meng and Zhang (2013),<sup>12</sup> a concentration of amygdalin below 5 mg kg<sup>-1</sup> is considered safe. Therefore, to make bitter apricot oil safe, environmentally friendly and sustainable, the amygdalin content needs to be lowered to a safe level. Various treatments have been reported to be effective in reducing the amygdalin content of bitter apricot kernels, thereby enhancing their usability in the pharmaceutical, cosmetic, and food industries. Zhang *et al.*, (2020)<sup>13</sup> revealed that ultrasonic treatment (700 W for 50 minutes) is an effective alternative method for reducing cyanide levels to as low as 4.65 mg kg<sup>-1</sup>. Further research is needed to develop a cost-effective, efficient and environment-friendly method to effectively lower the amygdalin content in apricot kernel oil to make it safe, expand its application in food and non-food industries, and make it a top alternative source of oil.

The current study aimed to investigate the efficacy of the hydro-treatment process in reducing the amygdalin content. The study was further extended to study the effect of hydro-treatment on the yield, physicochemical properties, total phenolic content, antioxidant activity and fatty acid composition of bitter apricot kernel oil.

## 2 Materials and methods

### 2.1. Materials

In this study, wild bitter apricot kernels were procured from the upper Himalayan region (with coordinates 33.86°N 75.29°E and an altitude of 2000 meters above sea level) of Jammu & Kashmir, India.<sup>14</sup> Wild bitter apricots were harvested and depulped. Their kernels were dried and stored for up to 3 months at 4 °C. Apricot kernels were manually cracked with a hammer, separated from shells, stored in airtight containers at 4 °C and processed within

a week. Apricot kernels were soaked in boiled water (~100 °C) for 2–3 min to remove the skin of kernels followed by drying at 50 °C for 2 h. The dried peeled apricot kernels were ground to a fine powder for oil extraction. All the chemicals used were of analytical grade.

### 2.2. Extraction of apricot kernel oil

Apricot kernel powder was mixed with hexane in a ratio of 1 : 10 and shaken for 1 h at 150 rpm at 35–40 °C. This process was repeated twice. Hexane was removed from the miscella by rotary evaporation under reduced pressure at 40–45 °C, followed by a short nitrogen purge for 10 min, to recover an oil termed control apricot kernel oil (AKO<sub>C</sub>).

### 2.3. Hydrotreatment to remove amygdalin

Amygdalin was removed from bitter apricot kernel oil using a detoxification method, conceptually adapted from Mirzaei and Rezaei (2019).<sup>15</sup> Briefly, miscella was mixed with distilled water in a ratio of 1 : 2 and shaken vigorously/vortexed for 15 min. Furthermore, the mixture was centrifuged at 6000g for 15 min followed by careful decanting of water. This process was repeated thrice. Hexane was removed from the treated miscella to obtain the oil, which was referred to as treated apricot kernel oil (AKO<sub>T</sub>).

The treated (AKO<sub>T</sub>) and control (AKO<sub>C</sub>) apricot kernel oils were stored at room temperature for further analysis.

### 2.4. Physicochemical properties

**2.4.1. Color analysis.** Color values *i.e.* *L\** (brightness), *a\** (green – red) and *b\** (blue – yellow) of control (AKO<sub>C</sub>) and treated (AKO<sub>T</sub>) apricot oil were assessed with a chromameter (CR 400, Konica Minolta). Color index (CI), chroma (*C\**), and hue (*h°*) angle values were measured using *a\** and *b\** values by putting them in the following equations:<sup>14</sup>

$$CI = \frac{1000 \times a^*}{L^* \times b^*}$$

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

$$h^{\circ} = \tan^{-1} \frac{b^*}{a^*}$$

**2.4.2. Refractive index and specific gravity.** The refractive index of control and treated AKOs was estimated using an Anton Paar refractometer (Abbemat 3200, Germany) at 27 °C. The specific gravity was determined using a pycnometer.

**2.4.3. Acid value.** The acid value of control and treated AKO was calculated according to AOAC (2006).<sup>16</sup> Briefly, a known weight (0.5 g) of sample was mixed adequately with ethanol (10 mL) and then, titrated with 0.1 N potassium hydroxide (KOH) solution using a phenolphthalein indicator until the pink color disappeared. The acid value was determined using the following formula:



$$\text{Acid value (mg KOH per g)} = \frac{56.1 \times \text{normality of KOH} \times \text{titre value of sample}}{\text{weight of sample (g)}}$$

where 56.1 is the equivalent weight of KOH.

**2.4.4. Iodine value.** The iodine value (IV) of AKOs was determined according to the method of AOAC (2006).<sup>16</sup> Briefly, AKO (1 g) was mixed with carbon tetrachloride (15 mL) and Wij's solution (iodine monochloride : glacial acetic acid 1 : 180) (25 mL) and kept in the dark for 30 min. After the reaction, 10% potassium iodide solution (15 mL) and distilled water (30 mL) were added to the reaction mixture with vigorous shaking. Then, the mixture was titrated with 0.1 N sodium thiosulfate solution using the starch indicator (3–4 drops) until the blue colour disappeared. Finally, the IV was calculated as follows:

$$\text{Iodine value (I}_2 \text{ g/100 g)} = \frac{(\text{BV} - \text{TV}) \times 12.69 \times N}{\text{sample weight (g)}}$$

where BV and TV are the titre value of the blank and sample, respectively.  $N$  = normality of  $\text{Na}_2\text{S}_2\text{O}_3$ , 12.69 = molar mass of iodine.

**2.4.5. Saponification value.** The saponification value of AKOs was evaluated using the AOAC standard method.<sup>16</sup> Briefly, 0.5 g AKO was mixed with 25 mL of 0.5 N alcoholic KOH in a round-bottom flask and refluxed. After one hour of refluxing, the mixture was titrated with 0.5 N HCl using phenolphthalein as an indicator until the mixture became colorless. Finally, the saponification value was evaluated using the following formula:

$$\begin{aligned} \text{Saponification value (mg KOH per g)} \\ = \frac{56.1 \times (\text{BV} - \text{TV}) \times \text{normality of KOH}}{\text{weight of sample (g)}} \end{aligned}$$

where BV and TV are the titre value of the blank and sample, respectively, and 56.1 is the equivalent weight of KOH.

**2.4.6. Oxidative stability test.** The peroxide value of AKO was measured using the standard method of AOAC (2006).<sup>16</sup> Briefly, a 0.5 g sample was mixed with a solution (acetic acid : chloroform mixture 3 : 2). 1 mL saturated potassium iodide solution and 30 mL distilled water were added to the mixture, followed by shaking for 1 min, and then titrated with 0.01 N sodium thiosulfate solution using the starch indicator until the blue colour disappeared. The peroxide value was measured using the following formula:

$$\text{Peroxide value (meq O}_2 \text{ per kg)} = \frac{(\text{TV} - \text{BV}) \times N \times 100}{\text{weight of sample (g)}}$$

where TV and BV = titre value of the sample and blank, respectively,  $N$  = normality of  $\text{Na}_2\text{S}_2\text{O}_3$

#### 2.4.7. Antioxidant activity

**2.4.7.1 DPPH radical scavenging activity.** DPPH scavenging activity was determined according to the method of Olszowy and Dawidowicz (2016),<sup>17</sup> with slight modifications. Briefly, 0.4 mL of the methanolic oil extract (0.7–3.5 mg mL<sup>-1</sup>) was mixed with 2.8 mL of 90 mM DPPH solution (prepared in 100% methanol). The mixture was vortexed for 1–2 min and kept in the dark for 30 min. The absorbance of the resulted mixture was measured at 515 nm using a UV-VIS spectrophotometer

(PerkinElmer, Korea). 100% methanol was used as a blank. The IC<sub>50</sub> (Inhibitory concentration) value was calculated based on the minimum sample concentration needed to inhibit 50% of the DPPH activity using the calibration curve.

$$\text{DPPH inhibition \%} = \frac{C - S}{C} \times 100$$

where  $C$  = absorbance of the control and  $S$  = absorbance of the sample.

**2.4.7.2 Ferric reducing antioxidant activity (FRAP).** The ferric-reducing activity of AKOs was assessed following the method of Chen *et al.*, (2016)<sup>18</sup> with slight modifications. Briefly, 2.5 mL of the methanolic oil extract (1–5 mg mL<sup>-1</sup>) was mixed with an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide, followed by incubation at 50 °C for 30 min. To stop the reaction, 2.5 mL of 10% trichloroacetic acid was added, followed by centrifugation at 10 000 rpm for 10 min. Then, 2.5 mL of the upper layer was mixed with an equal amount of distilled water and 0.1% ferric chloride solution. The absorbance of the mixture was measured at 700 nm using a spectrophotometer (PerkinElmer, Korea), with 100% methanol as the blank. The EC<sub>50</sub> (inhibitory concentration) value of AKOs was determined by calculating the effective concentration needed to exhibit 50% of the FRAP activity using the calibration curve.

**2.4.8. Total phenolic content.** The total phenolic content of AKOs was assessed following the method of Jin *et al.*, (2018),<sup>5</sup> with slight changes. Briefly, 2.5 g sample of AKOs was mixed with 2.5 mL of 80% methanol and vortexed for 60 min, followed by centrifugation at 3000 rpm for 10 min. This process was repeated twice. The upper methanolic layer (500 µL) was subsequently mixed with Folin-Ciocalteu (FC) reagent (2 mL) and 7.5% sodium carbonate solution (4 mL). The mixture was vortexed for 1 min and incubated for 30 min. The absorbance of the reaction mixture was evaluated at 765 nm with a spectrophotometer. Gallic acid was used as the standard and the results were reported as µg GAE (gallic acid equivalent) per g of oil.

#### 2.5. Amygdalin content

The amygdalin content of control and treated AKOs was measured using HPLC (Agilent 1260 Infinity II, G7115 1260 DAD WR), according to the method of Mirzaei and Rezaei (2019).<sup>15</sup> To extract amygdalin from AKOs, the sample (1 mL) was mixed with hexane (1 mL) and distilled water (2 mL) and vortexed for 30 min, followed by centrifugation at 5000g for 10 min. The upper layer of water was filtered using a 0.22 µm pore-size filter and transferred into an HPLC vial. Amygdalin was separated on an Agilent Column (C18, 4.6 × 100 mm, 4 µm) at 30 °C and detected at a UV wavelength of 214 nm. The mixture of water and acetonitrile (70 : 30, v/v) was used as a mobile phase. A 10 µL sample was injected and the flow rate was set at 0.7 mL min<sup>-1</sup>. The limit of detection (LOD) for amygdalin standard was 0.1 ppm, allowing reliable quantification of trace amounts of amygdalin in AKO.



## 2.6. Fatty acid composition analysis

The fatty acid profile of AKOs was determined by preparing fatty acid methyl esters (FAMES) using the method described by Joia *et al.*, (2025).<sup>14</sup> About 80 mg of AKOs was mixed with 3 mL of 6% methanolic HCL and heated in a water bath at 75–80 °C for methylation. After cooling, 2 mL of hexane was added to the mixture, followed by 1 min of mixing and 10 min of centrifugation at 5000g. Then, the upper layer was carefully collected and used for FAME analysis using GC-MS/MS (Agilent, 8890 GC, USA).

FAMES were analysed using an Agilent 8890 GC system (Agilent Company, US) equipped with a capillary column (100 m × 0.25 mm × 0.25 µm id), a flame-ionization detector (FID, temperature 275 °C) and a splitless injector (temperature 220 °C). The initial oven temperature was 80 °C with a holding time of 1 min, which increased at a rate of 5 °C min<sup>-1</sup> to 250 °C with a holding time of 5 min. The carrier gas used was helium (He) with a flow rate and split flow of 1 mL min<sup>-1</sup> and 50 mL min<sup>-1</sup>, respectively. The makeup flow and airflow of He were 25 mL min<sup>-1</sup> and 300 mL min<sup>-1</sup>, respectively. FAMES were identified by matching their retention times with the standard of FAMES under the same conditions. The calculation of fatty acids was done by measuring their relative area percentage.

## 2.7. FTIR spectroscopy

The infrared spectra of AKOs were recorded using ATR-FTIR (PerkinElmer, UATR Two, UK). A small quantity of oil sample (200 µL) was directly placed on the ATR crystal area. Scans were performed at an interval of 16 periodically, with a resolution of 8 cm<sup>-1</sup>, covering a 400–4000 cm<sup>-1</sup> spectral range. The ATR crystal was cleaned with isopropyl alcohol before each measurement.

## 2.8. Statistical analysis

All the experiments were performed in replicates. All the data were reported as mean ± standard deviation. Data were statistically analysed using a *t*-test ( $p \leq 0.05$ ) using Minitab-17 (Minitab Inc., State College, USA).

# 3 Results and discussion

Oil is the most significant factor affecting the overall commercial viability of seeds. The yield of apricot kernel oil (AKOs) ranged from 49 to 51%, with no significant ( $p > 0.05$ ) difference between AKO<sub>C</sub> (50.2%) and AKO<sub>T</sub> (49.8%). The consistency in oil yield after the hydrotreatment process indicated that the subsequent washing-centrifugation steps effectively separated the aqueous and oil phases, without miscella/oil loss. The high recovery of AKO<sub>T</sub> was attributed to the lipophilic nature of triacylglycerols, which resist aqueous partitioning.<sup>19</sup> No oil-in-water emulsion was observed, likely due to the low phospholipid content in apricot kernel, which reduces the interfacial stability.<sup>20</sup> Wang (2013)<sup>9</sup> also reported apricot kernel oil ranging from 44.0% to 55.17%. Matthäus and Özcan (2015)<sup>21</sup> and Chu *et al.*, (2013)<sup>22</sup> reported higher oil yields using Soxhlet extraction, with 53.4% in sweet apricot kernels and 58.78% in

*Amygdalus pedunculata*, respectively. These variations among the studies might be due to differences in apricot variety, kernel maturity, extraction method and also, to some extent, growing environmental conditions.

## 3.1. Physicochemical analysis

**3.1.1. Color.** The color of AKO<sub>C</sub> and AKO<sub>T</sub> samples was measured at room temperature. The visual appearance of AKO<sub>C</sub> was slightly dark yellow compared to AKO<sub>T</sub>. As shown in Table 1, the values of  $L^*$ ,  $a^*$ ,  $b^*$ , chroma and hue ( $h^\circ$ ) of AKO<sub>C</sub> were 49.64, -1.83, 13.74, 4.88 and 97.49°, respectively, and 49.53, -2.14, 13.70, 4.81 and 98.87°, respectively, for AKO<sub>T</sub>. AKO<sub>T</sub> exhibited significantly ( $p \leq 0.05$ ) higher  $a^*$  and lower hue values compared to AKO<sub>C</sub>. In contrast, the difference in  $L^*$ ,  $b^*$  and chroma values between AKO<sub>C</sub> and AKO<sub>T</sub> was non-significant ( $p > 0.05$ ). The  $h^\circ$  value of AKO fell in the second quadrant of the color wheel (90 refers to yellow and 180 to green color). The minor color differences between AKO<sub>C</sub> and AKO<sub>T</sub> may be due to the selective removal of hydrophilic pigmented compounds (*i.e.* polar phenolic and chlorophyll contents) during the aqueous washing steps. This treatment reduced the reddish undertone of oil without reducing lipophilic components like  $\beta$ -carotene.<sup>19,21</sup> Because of the mild and non-thermal treatment, the main pigments responsible for the yellowish color of oil remained unchanged, as carotenoids have very low water solubility and remained in the non-polar phase.<sup>19</sup> The unchanged  $C^*$  values further confirmed process gentleness, maintaining inherent saturation and optical clarity, as emulsion-free phase separation prevents light scattering from the leftover miscelles.<sup>20</sup> Jin *et al.*, (2018)<sup>5</sup> reported relatively lower  $L^*$  (38.8) and  $b^*$  values (8.8) and higher  $a^*$  values (4.2) for *Armeniaca sibirica* L. oil, while Durmaz *et al.*, (2010)<sup>23</sup> reported higher  $L^*$  (74.7) and  $b^*$  (74.1) values and lower  $a^*$  (-16.1) value for apricot kernel oil. Moreover, Uluata (2016)<sup>24</sup> reported  $L^*$  (73.8),  $a^*$  (-4.8) and  $b^*$  (19.5) values of cold-pressed apricot kernel oil in Hasanbey varieties. These variations may be due to the differences in oil extraction processes, cultivars and pigmentation levels in oil.

**3.1.2. Refractive index and specific gravity.** The refractive index (RI) and specific gravity (SG) of oil are governed by the degree of saturation and composition of oil, respectively. The RI and SG of AKOs ranged from 1.4682 to 1.4675 and 0.92 to 0.93, respectively. There was a non-significant ( $p \geq 0.05$ ) difference in the RI and SG of AKO<sub>C</sub> and AKO<sub>T</sub>. Similar findings were also observed by Bachheti *et al.*, (2012)<sup>26</sup> and Alpaslan and Hayta (2006)<sup>27</sup> for bitter apricot kernel oil (RI: 1.464 to 1.480 and SG: 0.87 to 0.93). Moreover, Sharma *et al.*, (2019)<sup>28</sup> reported comparable values for wild apricot kernel oil. Thus, the results indicated that hydrotreatment targets only polar compounds and impurities without affecting the non-polar matrix,<sup>19</sup> resulting in a stable refractive index and specific gravity for AKO. The unchanged RI values confirmed no oxidation, while the stable SG indicated unchanged molecular weight and density of the oil; this hydro-treatment process maintained the physicochemical integrity of the oil.

**3.1.3. Acid value.** Generally, the acid value measures the free fatty acids of oil. Oils with a high acid value are more prone





**Table 1** Physicochemical characteristics of untreated and treated wild apricot kernel oil (AKO) and comparison with FAO/WHO standard

Parameters	Untreated AKO (AKO <sub>C</sub> )	Treated AKO (AKO <sub>T</sub> )	FAO/WHO standard <sup>25</sup>
Yield, (%)	49 ± 1.17 <sup>a</sup>	51 ± 1.76 <sup>a</sup>	—
Visual appearance	Yellow	Pale yellow	—
Color values			
<i>L</i> *	49.64 ± 0.039 <sup>a</sup>	49.53 ± 0.210 <sup>a</sup>	
<i>a</i> *	−1.83 ± 0.130 <sup>a</sup>	−2.14 ± 0.076 <sup>b</sup>	
<i>b</i> *	13.74 ± 0.075 <sup>a</sup>	13.70 ± 0.717 <sup>a</sup>	
Chroma ( <i>C</i> *)	4.88 ± 0.012 <sup>a</sup>	4.81 ± 0.134 <sup>a</sup>	
Hue ( <i>h</i> °, degrees)	97.49 ± 0.493 <sup>a</sup>	98.87 ± 0.174 <sup>b</sup>	
Refractive index	1.468 ± 0.001 <sup>a</sup>	1.468 ± 0.001 <sup>a</sup>	1.46–1.47
Specific gravity	0.92 ± 0.006 <sup>a</sup>	0.93 ± 0.006 <sup>a</sup>	0.90–1.16
Acid value (mg KOH per g)	2.43 ± 0.32 <sup>a</sup>	2.43 ± 0.32 <sup>a</sup>	4.0
Iodine value ( <i>I</i> <sub>2</sub> g/100g)	93.14 ± 0.25 <sup>a</sup>	92.64 ± 0.67 <sup>a</sup>	80–106
Peroxide value (meq O <sub>2</sub> per kg)	1.50 ± 0.71 <sup>a</sup>	1.67 ± 0.58 <sup>a</sup>	<10
Saponification value (mg KOH per g)	192.50 ± 7.78 <sup>a</sup>	187.00 ± 11.0 <sup>a</sup>	181.40 ± 2.60
Total phenolic content (µg GAE per g)	27.28 ± 1.58 <sup>a</sup>	13.92 ± 2.85 <sup>b</sup>	—
Antioxidant activity			
DPPH (IC <sub>50</sub> , mg g <sup>−1</sup> )	6.84 ± 0.76 <sup>a</sup>	6.69 ± 0.89 <sup>a</sup>	
FRAP (EC <sub>50</sub> , mg g <sup>−1</sup> )	4.16 ± 0.37 <sup>a</sup>	4.26 ± 1.01 <sup>a</sup>	

Values are expressed as mean ± standard deviation. Values with different superscript letters in the same row are significantly different ( $p \leq 0.05$ ).

to rapid rancidity due to the hydrolysis during storage. As shown in Table 1, the acid value (AV) of AKO was 2.43 mg KOH per g of oil, with no significant difference between AKO<sub>C</sub> and AKO<sub>T</sub> ( $p > 0.05$ ). Under non-thermal treatment conditions, the ester linkages of fatty acids remained intact, indicating that hydro-treatment did not induce the hydrolysis of lipids, which resulted in a stable acid value of AKO after treatment. Moreover, partial removal of hydrophilic impurities (phospholipids and metals) can reduce the number of catalytic sites for hydrolytic activity, thereby enhancing the oxidative stability of oil.<sup>29</sup> Similar results were reported by Zhang *et al.*, (2022)<sup>19</sup> for rapeseed oil. The AV of AKO was found to be within the standard value for edible oils reported by FAO/WHO (<4.0 mg KOH per g)<sup>25</sup> and Hygienic Standard for Edible Vegetable Oil (2013)<sup>30</sup> (<3.0 mg g<sup>−1</sup>), confirming suitability for long-term storage and industrial use. Sharma *et al.*, (2019)<sup>28</sup> and Gupta *et al.*, (2012)<sup>31</sup> reported similar values of AV for *Balanites aegyptiaca* and wild apricot kernel oil, respectively. However, Bisht *et al.*, (2015)<sup>32</sup> reported a slightly high acid value (3.73 mg KOH per g) for cold-pressed wild apricot kernel oil, likely due to the lipase activity during kernel pressing.

**3.1.4. Saponification value.** Generally, the saponification value (SV) depends on the fatty acid chain length in the oil, thereby indicating the oil's average molecular weight. The SV of AKO (187.0–192.5 mg KOH per g) was closely similar to that of almond oil (186–195 mg KOH per g), indicating its potential suitability for industrial uses. The SVs for AKO<sub>C</sub> and AKO<sub>T</sub> were non-significantly ( $p \geq 0.05$ ) different, indicating that hydro-treatment did not affect ester integrity. Sharma *et al.*, (2019),<sup>28</sup> Mirzaei and Rezaei (2019),<sup>15</sup> Pavlović *et al.*, (2018)<sup>33</sup> and Shariatifar *et al.*, (2017)<sup>34</sup> also reported comparable SVs (183.3–195.5 mg KOH per g) for apricot kernel oil of different cultivars. As the length of fatty acids increases, SV decreases due to relatively low amounts of carboxylic functional groups per unit mass, in comparison to those rich in short-chain fatty acids. The increasing temperature causes the breakdown of fatty acids into

carbonyl compounds, decreasing the free fatty acid content and SV of oil.<sup>4</sup> The stable acid value indicated that no hydrolysis occurred during treatment. Furthermore, the ambient temperature of hydro-treatment was sufficient to prevent the thermal breakdown of triacylglycerols, thereby maintaining their molecular distribution and ensuring oil stability.

**3.1.5. Iodine value.** The iodine value (IV) is an indicator of oil oxidation stability, reflecting the overall degree of unsaturation. The higher IV of oil reflects greater susceptibility to auto-oxidation. As shown in Table 1, there was a non-significant ( $p \geq 0.05$ ) difference in the IV of AKO<sub>C</sub> (93.14 g I<sub>2</sub>/100 g) and AKO<sub>T</sub> (92.64 g I<sub>2</sub>/100 g), confirming that hydro-treatment did not cause any oxidation and isomerization of unsaturated fatty acids, requiring high temperature, metal catalysts and photosensitisers, which were absent in this hydro-treatment.<sup>19</sup> The IV of AKO was found within the prescribed range according to FAO/WHO standards (80–106 g I<sub>2</sub>/100 g)<sup>25</sup> and similar to that of almond oil (90–109 g I<sub>2</sub>/100 g).<sup>32</sup> The results are consistent with those reported by Shariatifar *et al.*, (2017)<sup>34</sup> in apricot kernel oil (90.3–103.9 g I<sub>2</sub>/100 g), but slightly lower than the IV of ultrasound-assisted extracted AKO (98.86–100.35 g I<sub>2</sub>/100 g), cold pressed and supercritical carbon dioxide extracted apricot kernel oil (102–103 g I<sub>2</sub>/100 g), and wild almond oil (94.0–98.0 g I<sub>2</sub>/100 g) as reported by Mirzaei and Rezaei (2019)<sup>15</sup> and Pavlović *et al.*, (2018).<sup>33</sup> The slight variation among the studies might be due to differences in oil extraction techniques, cultivars, and environmental conditions, which affect the oil composition.

**3.1.6. Peroxide value.** Peroxide value (PV) also indicates the oxidative stability of oils. As shown in Table 1, the PV of AKOs varied non-significantly ( $p > 0.05$ ) from 1.50 to 1.67 meq O<sub>2</sub> per kg of oil and remained under the limit (<10 meq per kg) prescribed by FAO/WHO.<sup>25</sup> Zhang *et al.*, (2022)<sup>19</sup> also reported stable PV in rapeseed oil after an aqueous washing step. The low and unchanged PV confirmed that the hydro-treatment process did not cause the oxidative degradation of unsaturated fatty acids, particularly linoleic acid, which are highly prone to



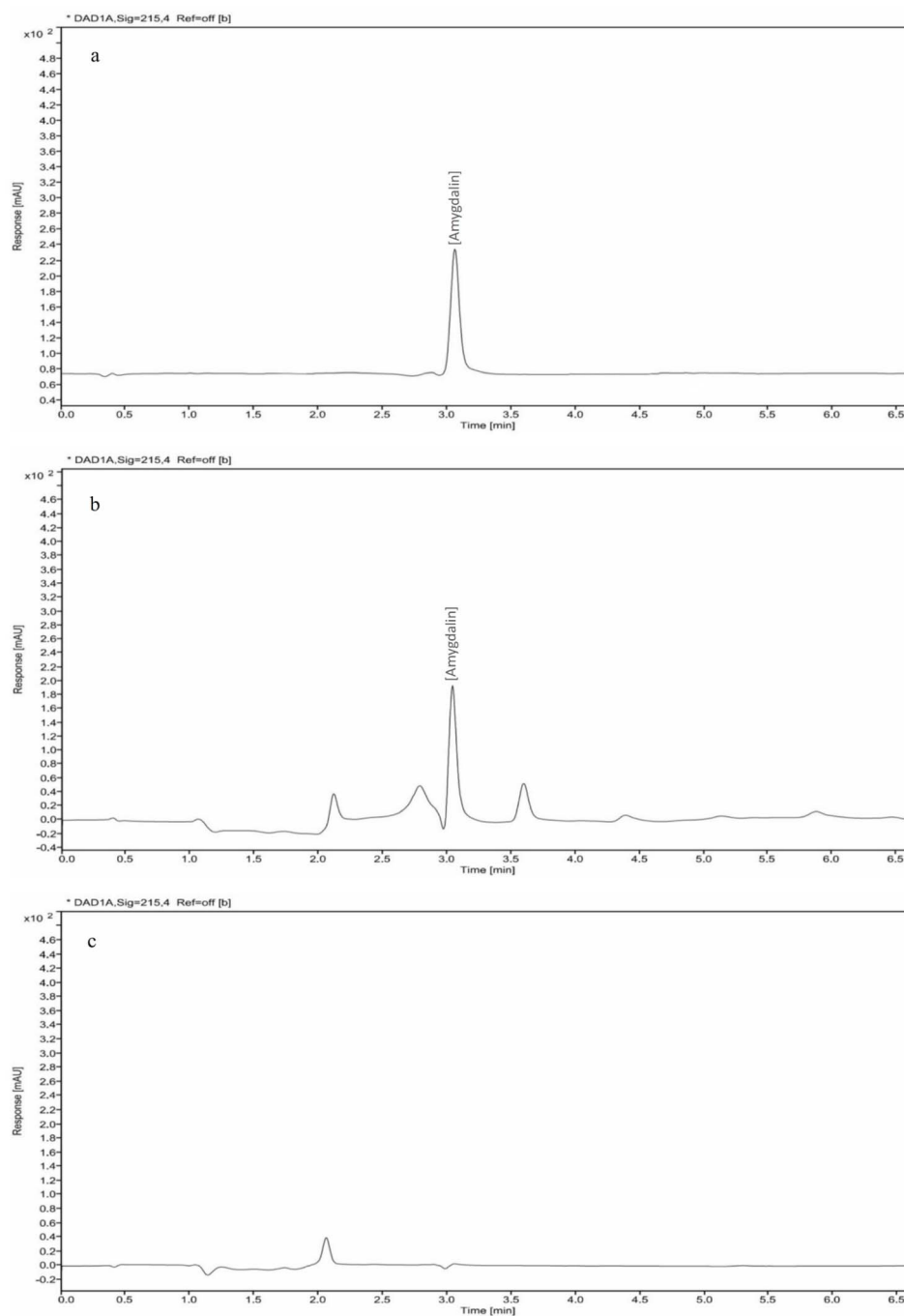


Fig. 1 HPLC chromatogram of Amygdalin standard (a), untreated (b) and treated (c) apricot kernel oil.

oxidation.<sup>19,25</sup> The oxidative stability of AKO<sub>T</sub> might be attributed to limited oxygen exposure, ambient temperature and removal of pro-oxidant impurities, such as metals. The lower PV indicated the good oxidative stability of AKO, similar to the findings for wild almond oil (0.70 to 2.36 meq O<sub>2</sub> per kg of oil), as reported by Mirzaei and Rezaei (2019).<sup>15</sup> Moreover, Pavlović *et al.*, (2018)<sup>33</sup> and Jin *et al.*, (2018)<sup>5</sup> obtained low PV values for Hungarian Best sweet AKO and *Armeniaca sibirica* kernel oil. In contrast, Gupta *et al.*, (2012)<sup>31</sup> and Sharma *et al.*, (2019)<sup>28</sup> reported high PV values for wild apricot kernel oil. It may vary due

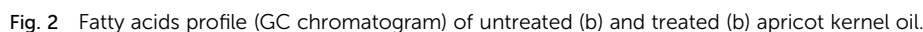
to the differences in extraction techniques, variety and environmental conditions. Yang *et al.*, (2013)<sup>35</sup> suggested that almond oil with a PV below 2.5 meq O<sub>2</sub> per kg is acceptable, whereas oil with a PV of  $\geq 5$  meq O<sub>2</sub> per kg or above produces a rancid flavor. Thus, a low and consistent PV of AKOPT reflects the superior oxidative stability and suitability for long-term storage. The unchanged, stable values of these parameters indicated that hydro-treatment is a non-degradative, quality-preserving technique.



Values are expressed as mean  $\pm$  standard deviation. Values with different superscript letters in the same row are significantly different ( $p \leq 0.05$ ).

Phenolic compounds play a significant role in enhancing the antioxidant capacity of oils. The total phenolic content (TPC) and antioxidant activity of AKOs are shown in Table 1. The results showed that AKO<sub>C</sub> had significantly ( $p < 0.05$ ) higher TPC (27.28 µg GAE per g) as compared to AKO<sub>T</sub> (13.92 µg GAE per g). The lower TPC in AKO<sub>T</sub> can be attributed to the polarity-based migration of hydrophilic phenolic compounds into the aqueous phase<sup>19</sup> during hydro-treatment. Zhang *et al.*, (2022)<sup>19</sup> also

As shown in Table 1, the DPPH scavenging activity ( $IC_{50}$ ) and reducing power (FRAP,  $EC_{50}$ ) of AKOs varied from 6.69 to 6.84  $mg\ g^{-1}$  and 4.16 to 4.26  $mg\ g^{-1}$ , respectively. There was no significant difference ( $p > 0.05$ ) in the values of these activities between AKO<sub>C</sub> and AKO<sub>T</sub>, indicating that the hydro-treatment



did not alter the antioxidant ability of AKO. Despite a reduction in TPC, non-phenolic lipid-soluble antioxidants remained unaffected mid-treatment, contributing to the stable antioxidant activity of AKO. In contrast, Szydłowska-Czerniak *et al.*, (2011)<sup>36</sup> reported a reduction in TPC and antioxidant activities of palm oil after aqueous washing (at 90–95 °C). Tavakoli *et al.*, (2019)<sup>37</sup> reported that the DPPH scavenging activity ( $IC_{50}$ ) of pistachio kernel oils varied from 12.7 to 15.9 mg mL<sup>-1</sup>. However, Fratianni *et al.*, (2021)<sup>38</sup> reported the higher antioxidant activity in cold-pressed seed apricot oil (DPPH  $IC_{50}$ , 10.01 µg mL<sup>-1</sup> and FRAP  $EC_{50}$ , 0.128 mg QE per g). Lower  $IC_{50}$  and  $EC_{50}$  values indicate higher antioxidant activity, suggesting that AKO has a relatively strong antioxidant potential. Variations among studies might be due to differences in cultivar and extraction techniques.

### 3.3. Amygdalin content

The amygdalin content in apricot kernel oils (AKO<sub>C</sub> and AKO<sub>T</sub>) is presented in Fig. 1. The amygdalin content in AKO<sub>C</sub> was 17.27 mg/100 g, whereas AKO<sub>T</sub> was amygdalin free. The amygdalin content in oil must be less than 5 mg kg<sup>-1</sup> to ensure safety.<sup>5</sup> Mirzaei and Rezaei (2019)<sup>15</sup> reported that hexane-extracted wild almond oil contained about 26 to 49 mg of amygdalin per 100 mL oil. Pavlović *et al.*, (2018)<sup>33</sup> revealed that the supercritical CO<sub>2</sub> extraction method was more effective in reducing amygdalin content (20 mg/100 g) compared with the cold-pressed method (40 mg/100 g). These findings suggest that the selected extraction method can effectively reduce the amygdalin content, enhancing the oil's safety and expanding its potential uses. Mirzaei and Rezaei (2019)<sup>15</sup> observed no differences in the amygdalin content between hot-pressed and cold-pressed wild almond oil. Zhang *et al.*, (2020)<sup>43</sup> reported that the ultrasonic treatment (700 W, 50 min) was the most effective method to reduce the cyanide content (4.65 mg kg<sup>-1</sup>) in bitter apricot kernel oil compared with microwave heating (8.33 mg kg<sup>-1</sup>) and water boiling (5.32 mg kg<sup>-1</sup>).

Amygdalin is a polar and hydrophilic compound with limited solubility in non-polar solvents like hexane.<sup>15</sup> During oil extraction, a portion of the amygdalin in apricot kernels is transferred to the oil.<sup>13,15,33</sup> However, during miscella washing with water, amygdalin migrates into the aqueous phase due to the polarity difference. This polarity-driven separation efficiently removes amygdalin, resulting in amygdalin-free AKO<sub>T</sub>.

### 3.4. Fatty acid composition

The effects of hydro-treatment on the fatty acid profile of AKO are shown in Table 2 and Fig. 2. The results showed a non-significant ( $p \geq 0.05$ ) difference in the fatty acid composition between AKO<sub>C</sub> and AKO<sub>T</sub>, indicating that hydro-treatment did not alter the fatty acid profile of AKO. The results indicated that the hydro-treatment process targets only polar impurities without affecting the non-polar matrix. This stability confirmed that hydro-treatment can safely detoxify the oil without affecting its nutritional properties. Zhang *et al.*, (2022)<sup>19</sup> also reported that the different aqueous treatments did not alter the fatty acid profile of rape seed oil. In AKOs, unsaturated and

saturated fatty acids varied from 91.18–92.18% and 7.48–7.57%, respectively. Furthermore, the unsaturated fraction of AKO consisted of monounsaturated fatty acids (MUFAs) (74.83–75.05%) and polyunsaturated fatty acids (PUFAs) (24.93–25.17%) with oleic and linoleic acid as the major fatty acids. AKO also contained omega-3 fatty acids (DHA: 0.93–1.14% and EPA: 0.17–0.18%). The high levels of oleic acid (67.60–68.55%) and linoleic acid (21.68–21.84%) impart both oxidative stability and essential fatty acid benefits, making AKO suitable for application purposes. Palmitic acid (5.24–5.36% of AKO) was prominent in saturated fatty acids, followed by stearic acid (1.64–1.67% of AKO). All these fatty acids comprised more than 98% of the total fatty acid content. Therefore, other fatty acids were detected in minor quantities, less than 1–2%. The fatty acid composition of AKO is consistent with earlier reports for almond oil and wild plum seed oil (*Prunus spinosa*) reported by Roncero *et al.*, (2021)<sup>39</sup> and Atik *et al.*, (2022).<sup>40</sup> Similarly, Manzoor *et al.*, (2012)<sup>41</sup> and Gupta *et al.*, (2012)<sup>31</sup> also reported that apricot kernel oil contains oleic (62.07–80.97%) and linoleic (13.13–30.33%) acids as the primary fatty acids, followed by palmitic acids (3.35–7.79%). Moreover, Zhou *et al.*, (2016)<sup>10</sup> and Jin *et al.*, (2018)<sup>5</sup> also reported a similar fatty acid composition in Longwangmo and Bitter (*Armeniaca sibirica* L.) apricot kernel oil, with oleic acid ranging from 70.29–72.40%, linoleic acid from 21.80–23.0% and palmitic acid from 4.45–4.87%.

### 3.5. FTIR spectra of oil

The FTIR spectra of apricot kernel oils are shown in Fig. 3. No noticeable change in the FTIR spectra of AKO after hydro-treatment was observed, indicating that hydro-treatment is a mild process that preserves the chemical structure and quality of the oil. The wavenumber regions and corresponding functional groups are summarised in Table 3. The characteristic band at 3005 cm<sup>-1</sup> corresponds to the *cis* double bond (=C–H) stretching, confirming the presence of unsaturated fatty acids, particularly oleic acid.<sup>42</sup> No shift/change in the intensity of this band indicated no alteration in IV or the degree of saturation of

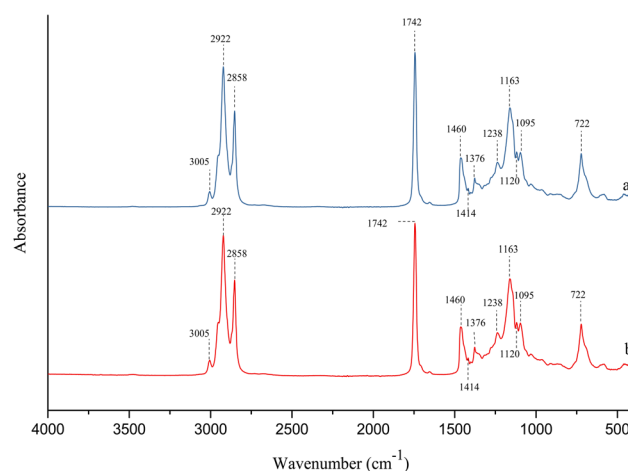


Fig. 3 ATR-FTIR spectra of untreated (a) and treated (b) apricot kernel oils in the spectral region (4000–400 cm<sup>-1</sup>).





**Table 3** Absorption peaks of treated and untreated apricot kernel oils in the FTIR spectral region (4000 to 400  $\text{cm}^{-1}$ ) along with their characteristic groups and mode of vibration

Wavenumber ( $\text{cm}^{-1}$ )	Characteristic group
3005	=C-H stretching ( <i>cis</i> double bonds)
2922	Asymmetric stretching of aliphatic groups ( $\text{CH}_2$ and $\text{CH}_3$ )
2858	Symmetric stretching of aliphatic groups ( $\text{CH}_2$ and $\text{CH}_3$ )
1742	Stretching of ester carbonyl group ( $\text{C}=\text{O}$ )
1460	-C-H ( $\text{CH}_2$ and $\text{CH}_3$ ) bending
1414	Rocking vibrations of aliphatic groups ( $\text{CH}_2$ and $\text{CH}_3$ )
1376	Bending vibrations of $\text{CH}_2$ groups
1238	Stretching vibration of -C-O groups
1163	Stretching vibration of -C-O groups
1120	Stretching vibration of -C-O group
1095	Stretching vibration of -C-O group
722	$\text{CH}_2$ bending (rocking) vibrations and -HC=CH- ( <i>cis</i> ) group of di-substituted olefins

oil after hydro-treatment.<sup>43</sup> Strong absorption bands at 2922 and 2858  $\text{cm}^{-1}$  were due to asymmetric and symmetric stretching of C-H aliphatic groups  $\text{CH}_2$ , respectively. No changes in the region of 3050–2800  $\text{cm}^{-1}$  confirmed the unchanged PV of AKO after hydro-treatment.<sup>44</sup> A sharp peak at 1742  $\text{cm}^{-1}$  corresponds to the ester carbonyl ( $\text{C}=\text{O}$ ) bond stretching. The consistent intensity and position of this band confirmed intact ester linkages, indicating no hydrolysis and oxidation of oil after hydro-treatment.<sup>43,45</sup> The additional peaks at 1460  $\text{cm}^{-1}$  and 1414  $\text{cm}^{-1}$  were due to the  $\text{CH}_2/\text{CH}_3$  bending and olefins rocking vibrations, respectively, whereas, the peak at 1376  $\text{cm}^{-1}$  was due to the bending symmetric vibrations of  $\text{CH}_2$  groups. The C-O stretching region (1238–1095  $\text{cm}^{-1}$ ) and 1742  $\text{cm}^{-1}$  ester band confirmed the unchanged SV *via* preserved glycerol ester content.<sup>43,45</sup> The peak at 722  $\text{cm}^{-1}$  was due to  $\text{CH}_2$  bending vibrations, characteristics of long fatty acids.<sup>43,46</sup> The FTIR spectra of AKOs exhibited a similar pattern to the previously reported FTIR spectra of extra virgin oil<sup>46</sup> and mustard oil.<sup>47</sup> Overall, the FTIR spectra of AKO showed no changes in AV, IV, PV, and SV of the oil, validating hydro-treatment as a safe, non-destructive detoxification method.

## 4 Conclusion

Bitter apricot kernel oil is generally undervalued due to the presence of cyanogenic glycosides (amygdalin), which can be effectively detoxified by hydrotreatment. Amygdalin is effectively removed using this process without affecting the yield or essential quality attributes (acid, saponification, iodine and peroxide values) in accordance with the FAO/WHO specifications. The oil still retains its fatty acid profile with considerable amounts of monounsaturated fatty acids. Notably, the post-treatment analysis confirms the complete removal of amygdalin, demonstrating the efficacy of the process in bitter apricot kernel oil and its safety for human consumption. As a result, hydro-treatment is an environmentally friendly and cost-effective approach for the utility of bitter apricot kernel oil in the food industry.

## Author contributions

Swati Joia: methodology, original draft preparation, investigation; Himashi Rao: investigation; Raashid Ahmad Siddiqui: review and editing; Tajendra Pal Singh: visualisation, supervision, conceptualisation, review and editing.

## Conflicts of interest

The authors have no conflicts of interest.

## Data availability

Data supporting this study are available from the corresponding author upon reasonable request.

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