

## CHARACTERIZATION OF SAFFLOWER OIL VARIETIES USING OPTIMIZED COLD PRESS METHODS

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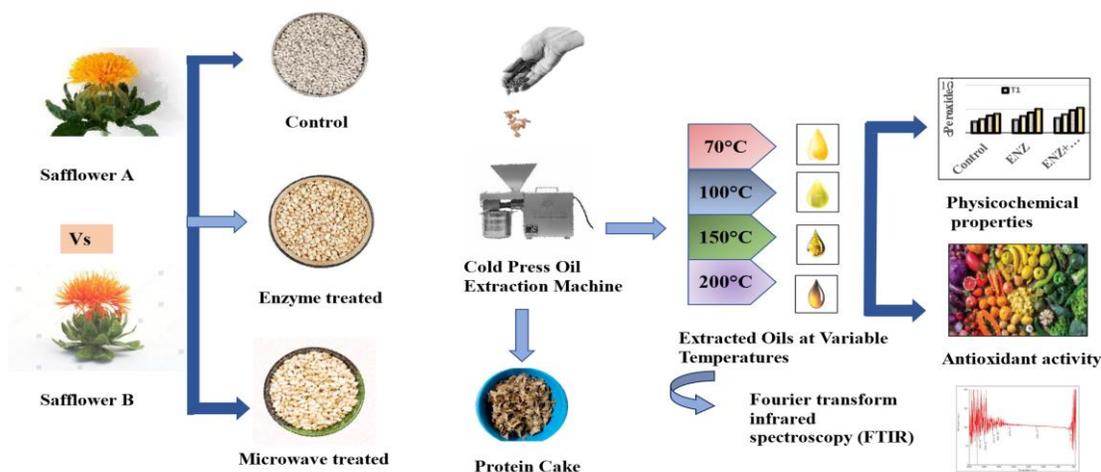
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### Abstract

Safflower oil finds extensive use in food, pharmacology, and industrial applications. The research study aimed for the appraisal of physico-chemical attributes of two safflower (*Carthamus tinctorius* L.) oil variants, (A and B), grown in different seasons and optimized growth conditions. The parameters were assessed using a variable temperature range of extracted oil (70°C, 100°C, 150°C and 200°C) while employing enzyme (pectinase) and microwave assistance methodologies. The investigated characteristics encompassed the yield (22.07-33.48%), density (0.998-0.257 g/cm<sup>3</sup>), refractive index (1.469-1.472), peroxide value (1.922-6.999 meq/kg), iodine value, (133-122 I/100g of oil), saponification value (202-181mg KOH /g of oil), acid value (0.11-0.31mg KOH /g of oil), as well as antioxidant activities including DPPH (2,2-Diphenyl-1-picrylhydrazyl) (1.32-0.57 IC<sub>50</sub>, ppm) and TPC (Total Phenolic Contents) (1.185-6.098 mg/g) further supported by Fourier transform infrared spectroscopy analysis. The yield, peroxide value, and antioxidant activities demonstrated a positive correlation except density, iodine value, and saponification value with the enzyme and microwave-assisted enzyme treatments applied. This correlation underscored the significant impact of extraction processes, influenced by temperature and the methodologies employed, on the properties of safflower oil. These findings provided valuable insights for optimizing the extraction and utilization of safflower oil across various industries.

**Keywords:** Safflower Oil, Cold Press Extraction, Enzyme Treatment, Temperature Effect, Yield, Density, Peroxide Value, Iodine Value, Saponification Value, Oxidative Stability, Fourier Transform Infrared Spectroscopy.

## Graphical Abstract



## INTRODUCTION

The market for vegetable oil is expected to develop with a Compound Annual Growth Rate (CAGR) of 5.14% from 2020 to 2025. The percentages of edible oil production and consumption are now 3.5% (8 Mt) and 7.6% (23 Mt), respectively. Imports will satisfy the existing (15 Mt) edible oil demand deficit [1]. Vegetable oils are one of the primary dietary elements in Pakistani culture that delivers the highest calories when compared to protein and carbohydrates. These oils are an important part of our diets and the economy because they are used as cooking oil, salad dressing, and frying oil [2]. Edible oils have multiple applications in traditional medicine, including the treatment of burns, edema, bronchitis, coughs, and colds. They are also vital to the body because they are the body's source of essential fatty acids, which are not synthesized there but are required for the integrity of cell membranes through diet [3].

Safflower (*Carthamus tinctorius* L.) is a significant edible oilseed crop whose potential for oil and protein cakes has not yet been completely realized [4]. As a member of the Compositae] family, it is an extensively cultivated oilseed crop in China, India, the United States, and Mexico [5]. It is a useful crop for arid agricultural areas because of its capacity to withstand drought. The low-rainfall regions of KPK and Punjab in Pakistan are the most ideal for its production [6]. Due to its tap root system, this plant grows well in dry climates or areas that occasionally experience rain. Safflower plants can grow to a length of 60 cm. One of the greatest places in the world to grow safflowers is in the arid region of Central Asia and China's Xinjiang Autonomous Region, which has a distinct climate with significant temperature changes between day and night, drought, and lots of sunshine [7]. It is getting more and more common to create unique safflower cultivars with more seed oil. Another area of research for such kinds of oils is a healthy fatty acid profile with high levels of mono- and polyunsaturated fatty acids [8].

Production of safflower has been increasing over the past few years, as shown by a 4.90% annual increase in agricultural land. The average global yield of safflower seeds has varied from 805 to 872 kg ha<sup>-1</sup>, with an annual increase rate of 0.97%. Despite being a small crop, global safflower production climbed by 5.60% annually in 2014 to 867,659 tonnes. 93% of this crop's entire production comes from two primary producing regions: Asia and America [9].

Safflower oil is currently employed mostly in the food industry due to the higher levels of mono and polyunsaturated fatty acids. Additionally, safflower oil has a high energy content and includes important substances such as carotenoids, phospholipids, phytosterols, and tocopherols [10]. Safflower oil has been discovered to provide several health advantages in recent studies. Safflower oil's balanced fatty acid profile has been shown to inhibit rat fat accumulation when compared to a diet high in beef tallow [11]. Clinical studies have shown that safflower oil's conjugated linoleic acid effectively decreases body weight and fat tissues [12]. Safflower oil has also been shown to be advantageous for treating insulin resistance brought on by fat [13]. However, it can be used as biodiesel either by itself or in combination with other oils. According to certain studies, safflower and castor oil are combined to create biodiesel, which provided a decreased viscosity as a result [14]. In Korea, safflower seeds that are high in  $\alpha$ -linoleic acid and unsaturated fatty acids are also utilized in clinical therapies for rheumatoid arthritis and osteoporosis. Safflower seeds are also frequently consumed as vegetable oils in the United States and Europe. Despite substantial reporting on the biological functions of this plant, there is little evidence of its inhibitory effects on melanogenesis (synthesis of melanin pigments) [15].

Techniques for extracting oil have pinched a lot of consideration as a means of increasing production. Mechanical, solvent, and supercritical CO<sub>2</sub> extraction techniques have been used to extract oil. Solvent residue in oil can be successfully fixed with supercritical CO<sub>2</sub> fluid extraction. However, because of the comparatively high equipment investment, it is not appropriate for the edible oil processing business as it exists today. Cold pressing is the process of producing oil by mechanically breaking down the oil cells in plants without using heat, thereby releasing the oil from the raw materials [16]. Oil composition varies depending on the technique and methodology utilized to obtain it [17]. Historically, safflower oil has been extracted using a method called "ghani" (primarily in Indian regions), which combines a pestle and mortar [18].

One of the simplest methods for extracting oil from seeds without the use of a solvent is cold press extraction. A quick decorticating approach is routinely utilized to reduce the energy expense of the extraction process [19].

The physical process of cold pressing applies pressure without heating and doesn't affect the oil or active ingredients. Apart from the standard features of the oil production process, this method can also enhance the oil's quality, prevent the presence of harmful substances like trans fatty acids and oil polymers resulting from high processing temperatures, and preserve the oil's active ingredients [20]. The method described not only

enhances nutritional value, maintaining active ingredients in the pressed cake, but also prevents hazardous material residues from chemical refining additives. It accelerates processing time and reduces production costs, and the simultaneous production of high-quality oils and macromolecular nutrients from oil crops. Consumer demand for edible oil extraction that is safe, nourishing, and solvent-free is rising as health consciousness increases. [21].

Enzymatic extraction stands out as a primary method for augmenting the extraction of bioactive constituents from oil. To achieve immediate separation of oil and protein cake, Some researchers coupled aqueous extraction with enzymes to create a novel extraction method called the aqueous enzyme method (AEE) to increase the oil yield of aqueous extraction [22]. Although pre-processing methods such as microwave heating ultrasound treatment supercritical extraction techniques [17] enzymatic extraction techniques, ultrasonic extraction, and combinations of these methods [23] can improve production, and quality and produce cold-pressed oils that are enhanced with bioactive components. Cold pressing of oilseeds usually avoids thermal treatment [20].

This research trends to produce safflower seeds oil by cold press extraction, microwave mechanical extraction, and enzyme microwave mechanical extraction. Furthermore, the recommendation of the more valuable variety of safflower oil is decorated in terms of its oxidative stability by evaluating their physicochemical parameters. Growing safflower seed varieties as an alternative of imported edible oil production is a positive contribution to saving the economy of agricultural countries such as Pakistan. For this, a thorough evaluation of the recently published literature is necessary, and the study has tried to address many points.

## 2. MATERIALS AND METHODS

### 2.1 Sample Preparation

Two different safflower seed varieties (designated as A and B) were obtained from the agronomy department of the University of Agriculture Faisalabad. The oils were obtained by cold press machine at 50°C, 100°C, 150°C and 200°C. The obtained oil was stored in brown bottles for further analysis. Yield, density, refractive index, peroxide value iodine value, and saponification number, acid value, and antioxidant activity of the control, microwave treated seeds, and combined effect of enzyme and microwave treatment upon oils were determined by adopting various standard methods.

### 2.2 Determination of Yield of Extracted Safflower Oil

Weighing was used to ascertain the oil's final weight, which was then represented as the percentage of oil content [24].

$$\text{Oil content (\%)} = \frac{\text{Weight of oil extracted}}{\text{Weight of seed sample}} \times 100$$

### 2.3 Determination of Density and Refractive Index of extracted oil

Density and refractive index of extracted oil were determined as per the methods given by the standard method described in [25].

### 2.4 Determination of Peroxide Value (PV) of extracted oil

The oil sample weighed 5.0 g (weight by weight) and was put into a 250 mL flask. Glacial acetic acid/chloroform (3:2, v/v) solutions were added and agitated for 30 minutes. After inserting a stopper, the flask was shaken for a minute and then left in the dark at 15 to 25 °C for about 5 minutes. The liberated iodine was titrated with 0.01N Na<sub>2</sub>SO<sub>4</sub>, employing starch as an indicator, after 30 mL of distilled water had been added into it. The PV was computed using the reported literature [26].

### 2.5 Determination of Iodine Value (IV) of extracted oil

The iodine value was calculated using the AOCS standard procedure 1992 [27]. A 250 mL iodine flask containing a 0.1–0.3 g of oil sample was filled with 25 mL of carbon tetrachloride (CCl<sub>4</sub>) solution. Later on, both were mixed with 25 mL of Wij's solution. The mixture was let to stand in the dark for 30 minutes. 150 mL of distilled water was also added into it after adding 10 mL of the 15% potassium iodide (KI) solution and given it a good shake. The iodine value was measured by titrating with a standard 0.1 N sodium thiosulphate solution until the blue hue disappeared. A few drops of starch solution served as the indicator. A blank determination was conducted in addition to the sample.

$$IV = \frac{(B-S) \times N \times 12.7}{\text{Weight of sample (g)}}$$

B = volume of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O used for blank

S = Volume 0.1 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O used for sample

N = Normality of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O

### 2.6 Determination of Saponification Value (SV)

The saponification value serves as an indicator of safflower oil quality, determined by the method reported by literature [28].

### 2.7 Determination of Acid Value

The acid value of oil samples was carried out by following the standard IUPAC method 1992 [26].

### 2.8 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Safflower extracted oil samples (at 100 and 200 °C) of both varieties (A and B) were subjected to FTIR analysis using Fourier Transform Infrared Spectrophotometer (Spectrophotometer-3000 SN: ZRMFIE06). The results were obtained from the Hi-tech laboratory of Government College Women University Faisalabad.

## 2.9 Evaluation of Antioxidant Activity

### 2.9 (a) Determination of Total Phenolic Content (TPC)

The quantity of TPC was ascertained using the Folin–Ciocalteu reagent (FCR) method, as detailed by literature [29]. 0.5 mL of the 2.0 N FCR, 0.05 g/5 mL of oil extract solution, and 7.5 mL of deionized water were combined. For 10 minutes, the mixture was left at room temperature. Later on, 1.5 mL (15%) sodium carbonate (w/v) was added into it. After 20 minutes of heating at 40 °C in a water bath, the mixture was cooled in an ice bath until the Prussian blue coloration appears. At 755 nm, the absorbance was finally measured with spectrophotometer (DB-20. No. 6422004 UV-VIS spectrophotometer).

### 2.9 (b) Determination of DPPH Radical Scavenging Assay

The ability of the oil extracts to scavenge the stable radical 2,2  $\gamma$ -diphenyl-1-picrylhydrazyl was used to determine their antioxidant activity. The DPPH assay was carried out according to the given instructions by literature [30]. To create a final volume of 4 mL, the oil extract samples (0.5 to 15.5  $\mu\text{g mL}^{-1}$ ) were combined with 1.0 mL of a 90  $\mu\text{M}$  DPPH solution. Following an hour of incubation time at room temperature, the absorbance of the solution and of the blank's were assessed at 515 nm. Butylated hydroxytoluene (BHT) was used as a positive control. Three replicates of each sample were created. Using a spectrophotometer to measure absorbance at 515 nm, the discoloration of solution was ascertained spectrophotometrically. Scavenging of free radical (DPPH) (%) was determined by the following formula:

$$I (\%) = 100 \times (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}})$$

Where  $A_{\text{blank}}$  is the absorbance of the control reaction mixture excluding the test compounds, and  $A_{\text{sample}}$  is the absorbance of the test compounds.

## 3. RESULTS AND DISCUSSION

### 3.1 Percentage Yield Oil of Safflower Seed Varieties

Experimental data of oil yield is given in Table. 1 and Figure 1-2. The observed yield was 22.07% -31.73% for variety A and 22.82%-33.48% for variety B. Results represented that there is an overall increase in the yield of safflower extracted oil samples while the temperature of the cold press extraction machine rises as: T1(70°C) <T2(100°C) <T3(150°C) <T4(200°C). Following the temperature range 70°C-200°C, yield obtained by control, enzyme, and microwave-assisted enzyme samples of variety A was 22.07-26.08%, 23.98-28.46,% and 27.09-31.73% respectively. Moreover, when comparing the yield obtained by different treatment methods such as control, enzyme-assisted, and microwave-assisted enzyme on variety B samples, distinct trends emerged. The yields, expressed as percentages, showcased a progressive enhancement across these treatment modalities. Specifically, the yield ranges for variety B are as follows: Control: 22.82-%26.83% Enzyme-assisted: 24.73% to 31.21% Microwave-assisted enzyme:

27.34% to 33.48%. When the results of both treatments were compared, microwave treatment as well as microwave and pectinase enzyme treatment showed a pronounced increase in the oil yield of safflower oil in comparison to the control. While comparing the yield of both varieties A and B, variety B given out a higher yield of 33.48 % at 200°C under the microwave-assisted enzyme treatment. Although there was an increase in yield with the temperature rise as well for control and enzyme treatments. The observed results are aligned with those obtained by literature <sup>[31]</sup> stated that initially, the safflower seeds had an oil content of 34.1 g per 100 g in their raw state (without any treatment). However, after undergoing different processing techniques such as roasting and boiling, the oil content increased to more than 36.0 g per 100 g in the extracted samples. This indicates that the processing techniques, specifically roasting and boiling, had a significant impact on increasing the oil content of the safflower seeds. The alignment of the current study's results suggests consistency and validation of the findings across different studies or experiments, reinforcing the reliability of the observed effects of processing techniques on safflower seed oil content.

### 3.2 Density of Extracted Safflower Oil Varieties

One of the distinct physical characters of oil is density. Two safflower oil varieties (A and B) were subjected to different treatment conditions. Treatment conditions included temperature variations (70°C, 100°C, 150°C and 200°C), enzyme exposure, and microwave-assisted enzyme treatment using pectinase enzyme. As the temperature increased, the density of safflower oil decreased as shown (Table. 2) (Figure 3-4). The density for variety A was found to decrease from 0.991- 0.331 g/cm<sup>3</sup> and 0.998-0.257 g/cm<sup>3</sup> for variety B. Across the temperature range of 70°C to 200°C, the density values obtained for variety A samples vary depending on the extraction method employed. Specifically: control: 0.991 to 0.239 g/cm<sup>3</sup> Enzyme-assisted: 0.976 to 0.342 g/cm<sup>3</sup> Microwave-assisted enzyme: 0.965 to 0.331 g/cm<sup>3</sup>. Furthermore, noticeable patterns showed up when the yields from the three different treatment methods control, enzyme-assisted, and microwave-assisted enzyme for the variety B samples were examined. The control method yields densities ranging from 0.998 to 0.509 g/cm<sup>3</sup>. Enzyme-assisted extraction results in densities spanning from 0.981 to 0.332 g/cm<sup>3</sup>. Microwave-assisted enzyme extraction demonstrated densities ranging between 0.976 to 0.257 g/cm<sup>3</sup>. This inverse relationship indicates thermal expansion, where higher temperatures lead to increased molecular movement and thus reduced density<sup>[32]</sup>. In comparison to treatments without the enzyme, the addition of the pectinase enzyme to the microwave-assisted treatment caused a little drop in the density of safflower oil. This implies that the oils' molecular structure may have been slightly modified by the enzymatic activity.

### 3.3 Refractive Index of Safflower Oil Varieties

The refractive index (RI) of oil signifies the ratio of the speed of light at a specified wavelength to its velocity within the oil medium. These RI values play a key role in identifying and classifying different types of oils. It often falls short in offering comprehensive data for the quantitative identification of a pure analyte. However, they

serve as valuable tools in verifying oil contamination or adulteration. The primary application of the refractive index lies in quantifying the alteration in unsaturation during the hydrogenation process of fats or oils. In the case of safflower oil, its refractive index is measured as 1.469 for variety A and 1.472 for variety B shown in (Table. 3) (Figure 5-6). Although the impact of microwave and enzyme assistance doesn't directly alter the refractive index value, it can expedite the measurement process without altering the inherent properties of the substance being analysed our findings are relatable to the results reported by research studies [32].

### 3.4 Peroxide Value of Safflower Oil Varieties

Peroxide value of different varieties of safflower at various temperatures (70°C, 100°C, 150°C and 200°C) was performed. It was observed that there was a regular increase in the peroxide value of different varieties of safflower oil with the temperature rise. Data are provided in (Table. 4) and (Figures 7 and 8). According to the findings discussed, the heating process initially causes a rise in the peroxide index to its maximum value in the control of variety A (1.922-5.888 meq/kg) and (2.122-4.888 meq/kg) in the control of variety B. Upon enzyme assistance, the peroxide index exhibits a range of values for both variety A (ranging from 2.691 to 5.895 meq/kg) and variety B (ranging from 2.309 to 6.095 meq/kg). When subjected to microwave-assisted enzyme (ME) treatment, the peroxide values for variety A exhibit a range from 3.911 to 6.998 meq/kg, while variety B displays a dissimilar range from 3.109 to 6.999 meq/kg. Variety B gives out a maximum 6.999 (meq/kg) peroxide value which was found under the Microwave-assisted Enzyme treatment at 200°C. In the case of safflower oil, during the initial stages of heating the peroxide value increases. This elevation is attributed to the formation of hydroperoxides derived from the oxidation of unsaturated fatty acids present in the oil. As heat is applied through microwave assistance, oxygen reacts with these unsaturated fatty acids liberated more by treatment with pectinase enzyme, leading to the formation of hydroperoxides as primary oxidation products as a result we found maximum peroxide value under the ME treatment. The peroxide value values during the course of two applied treatments enzyme-assisted and microwave-assisted enzyme were found to have increased by more than 5.077% in our study's results. Our results are relatable to [33] found that the heating of the oil causes the rise of the index of peroxide at the start of cooking to an extreme value and then there is a decrease. Highly unsaturated oils are oxidized more rapidly than less unsaturated oils. The partial pressure of oxygen in the oil's headspace affects the amount of oxygen present. When the partial pressure of oxygen in the headspace is higher, more oxygen is dissolved in the oil, leading to a faster oxidation process, a temperature, time, and light-dependent, quantifies the degree of primary oxidation (rancidification) of oils. Oil rancidity can result in the production of potentially hazardous substances associated with long-lasting health issues like cancer, neurological, and heart diseases. Analogous to saturated oils, highly unsaturated oils are far more exposed to oxidation. The study contributes to a deeper understanding of safflower oil stability and guides consumers, producers, and researchers in making informed decisions related to its storage, utilization, and potential health benefits. The peroxide value of safflower oil

has implications for its potential health benefits. Low peroxide values signify minimal oxidative damage to the beneficial unsaturated fatty acids, promoting the oil's positive impact on cardiovascular health, inflammation, and oxidative stress. Incorporating safflower oil with optimal peroxide values into a balanced diet may contribute to overall well-being. Elevated peroxide values indicate advanced oxidative deterioration and may lead to off-flavors, reduced nutritional content, and potential health risks. Monitoring peroxide values in safflower oil is essential to ensure consumer safety, maintain product integrity, and comply with regulatory standards. Furthermore, understanding the impact of peroxide values on safflower oil's sensory attributes and nutritional composition aids in optimizing its use in culinary and food manufacturing applications.

### 3.5 Iodine Value of Extracted Safflower Oil Varieties

Iodine values for oil varieties A and B were recorded that were extracted at different temperatures (70°C, 100°C, 150°C and 200°C). This calculation showed a decrease in iodine values. In particular, the iodine value dropped from 133 to 122 IV/100g of oil for variety B and from 131 IV/100g to 122 IV/100g of oil for variety A. There was a gradual decrease in the iodine value depending upon the treatments applied with the rise in temperature during extraction. It was evident from the experimental data as the temperature of extraction rose to 200°C the iodine value of the control sample of variety A and B dropped from 126 IV/100g to 122 IV/100g of oil and 128 IV/100g to 122 IV/100g of oil respectively.

The iodine value of variety A under microwave and enzyme-assisted treatment drops to 125, five points from the initial value of 131 IV/100g observed at 70°C. Furthermore, while talking about the variety B at 200°C iodine value 133 IV/100g was observed dropping 3 points from initial 128 IV/100g. Iodine value suggested that variety B of safflower oil is somewhat less oxidized by two points in comparison to our variety A. These values were analysed when both A and B were treated to microwave alone and microwave enzyme (ME) assisted extraction.

The Variety A oil had the fastest loss of unsaturation of oils heated at 200°C compared to sample B (Table. 5) and (Figure 9-10). The iodine value of an oil is influenced by the degree of unsaturation, as unsaturated fatty acids can react with iodine to form iodine compounds. As temperature increases, the rate of reactions also typically increases. Several studies have investigated the effect of temperature on the iodine value of different oils, including safflower oil. The iodine value of safflower oil decreased with increasing temperature, which was attributed to the accelerated oxidation and polymerization of unsaturated fatty acids [34]. The iodine values of oils with the treatment of enzyme and microwave enzymes (ME) were compared with the untreated safflower oils varieties and out of this, it was found that there was a decrease in iodine value in all the oils after they were treated with enzyme and microwave. This was in agreement with the finding [35]. That there is a decreasing trend in the iodine value of the oil during the temperature rise. The decrease in iodine value with the treatment of enzyme and microwave could be attributed to the changes in fatty acids taking place with roasting in the microwave.

### 3.6 Saponification Value (SV) of Extracted Oil Varieties

The saponification value provides insights into the average molecular weight of fatty acids in an oil. It is determined by the amount of alkali required to saponify a known quantity of fat or oil. In our experimental section with the temperature rise, there is a regular decrease in the saponification value of the oil varieties from (199-194, 197-190, 190 - 184 mg KOH /g of oil) of variety A, (202-192, 201-193 198 to 193 mg KOH /g of oil) of variety B for control, enzyme, and enzyme assisted microwave extraction respectively. When the temperature increases from 70°C to 200°C presented in Table. 6 and Figures 11-12. Temperature plays a role in the rate of hydrolysis and saponification reactions. A study on different vegetable oils, including safflower oil, indicated that saponification values decreased with increasing temperature, mainly due to increased hydrolysis of ester bonds at higher temperatures<sup>[32]</sup>.

### 3.7 Acid Value of Extracted Safflower Oil Varieties

The Acid value serves as an indicator of the concentration of free fatty acids present in oil. A higher acid value signifies an elevated level of free fatty acids, indicative of decreased oil quality. Results demonstrated that the acid value reflects the free fatty acid content resulting from enzymatic activity in the oils (Table. 7) and (Figure 13-14). Safflower oil's control varieties A and B exhibited an acid value of 0.19-0.22 mg KOH/g and 0.20-0.24 mg KOH/g respectively. When subjected to enzyme treatment, the results showed a notable rise in acid values for both Variety A, increasing from 0.23 mg KOH/g to 0.28 mg KOH/g, and Variety B, escalating from 0.25 mg KOH/g to 0.31 mg KOH/g. This increase may be attributed to the hydrolysis caused by the enzyme. Moreover, samples treated with microwave-assisted enzyme extraction exhibited a statistically significant reduction in acid value, decreasing from 0.16 mg KOH/g to 0.11 mg KOH/g for variety A and 0.19 mg KOH/g to 0.16 mg KOH/g for variety B. This decline in acid value can be attributed to the thermal pretreatment causing pectinase inactivation<sup>[32]</sup>.

### 3.8 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra of variety A and B at 100°C and 200°C are shown in Figure. 15a, 15b, and 16a, 16b respectively. The major peaks in these spectra appear from C-H stretches of triglyceride structures between 3007 and 2760  $\text{cm}^{-1}$ , C=O stretching vibrations at 1750  $\text{cm}^{-1}$ , CH<sub>2</sub> and CH<sub>3</sub> scissoring bindings at 1460 and 1163  $\text{cm}^{-1}$ , and CH<sub>2</sub> rocking modes at 726  $\text{cm}^{-1}$ . The VIP values for the model indicated that wavenumbers responsible for differentiation fit the well-known absorption bands (1815–659, 3146–2816, 3391–3382, 3631–3530  $\text{cm}^{-1}$ ) of a general oil spectrum. Peaks around the 3600–3500  $\text{cm}^{-1}$  region could be attributed to –OH stretching while the 3300–2800  $\text{cm}^{-1}$  region is associated with –CH stretching. The C-O stretch takes place at 1800–1700  $\text{cm}^{-1}$  and 1200–650  $\text{cm}^{-1}$  is the fingerprint region<sup>[36]</sup>. The absorbencies of safflower oil varieties A and B at 100°C exhibited notable changes when compared to oil subjected to extraction at 200°C indicating a distinct impact of extracting temperature on the oil. Particularly, the band near 3006  $\text{cm}^{-1}$  experienced a noticeable decrease in intensity at 200°C as depicted in Figures

and. This decrease suggests a reduction in the degree of unsaturation because of the oxidation process. Our results are correlated with <sup>[31]</sup> suggesting that when compared to the control oil, safflower oil derived from roasted safflower seeds was preserved for three days and the first day under oxidative conditions. The band near  $3006\text{ cm}^{-1}$  decreased indicating a decrease in the degree of unsaturation because of the oxidation process. The peak intensities (absorbance values) of control oil were altered in comparison with oil subjected to roasting, indicating a clear effect of roasting temperature on the oil.

### 3.9 DPPH activity of safflower oil varieties

At 70, 100, 150, and 200 antioxidant activities for variety, A were found to increase from 0.91-0.57 IC<sub>50</sub>, ppm and 1.32-0.68 IC<sub>50</sub>, ppm for variety B given in (Table 8) and (Figure 17, 18). Low to high temperatures originate a vibrant increase in DPPH activity in the presence of enzymes from (0.86-0.59 IC<sub>50</sub>, ppm for variety A) and (1.29-0.87 IC<sub>50</sub>, ppm for variety B). Under microwave-assisted enzyme treatment variety A exhibits maximum antioxidant activity 0.57 IC<sub>50</sub>, ppm at 200°C when compared to both ME-assisted seed oil varieties A and B at temperatures 70°C, 100°C and 150°C. These findings support those of <sup>[37]</sup> who demonstrated that the high oxidative stability of oil derived from roasted safflower seeds was caused by enzymatic reaction compounds formed during the heating process; these substances are highly polar because of active radicals<sup>[38]</sup>. The process of heating and microwave-assisted enzyme treatment in oilseeds involves a slight interaction between the creation of new Maillard reaction products, which possess antioxidant properties, and the thermal disruption of naturally occurring antioxidant compounds. Roasting produces components of the Maillard reaction, which boosts antioxidant capacity; however, this impact may be countered by the heat and enzyme destruction of antioxidants that are already present. Thus, knowing and controlling this dynamic equilibrium is essential to figuring out oilseeds that have been roasted in terms of their ultimate antioxidant profile. The creation of novel molecules with antioxidant qualities during heating, such as melanoidin during Millard reactions, maybe the cause of the rise in antioxidant activity as measured by the roasting temperature of safflower seeds. The effects of pre-treating goldenberry seeds with enzymes on their capacity to scavenge oil-derived DPPH radicals have been studied by other writers <sup>[39]</sup>. The study found that after 30 minutes, the inhibition of DPPH radicals was 53.5 and 49.7% for untreated goldenberry seeds and enzyme-pretreated seeds (Cellulase EC, Pectinase L 40 (1:1), 50 °C, pH: 4.3, enzyme concentration: 2% (w/w), 2 h). This suggests that the enzyme-treated seeds had a 7% higher capacity to scavenge DPPH radicals than the oil from the untreated seeds. These results imply that the microwave power, pulse intensity, and exposure duration affect the antioxidant activity of seed oil.

### 3.10 Total Phenolic content (TPC)

Natural bio-actives acting as antioxidants called phenolic compounds are found in large quantities in natural oils and have a strong correlation with the flavour and stability of the oil. Accordingly, the ability of rapeseeds to be pretreated in a microwave for two minutes at 800 W to increase the amount of oil phenolic compounds was assessed. Depending

on the cultivar, these chemical contents show variations in various oils [40]. The results summarized in the paper show that extracted oils at 70-200°C caused a significant increase in phenolic compound content of oil from 1.185-5.164 for variety A and 1.985-6.098 for variety B. Maximum value 6.098 of variety B was recorded at 200°C under the microwave-assisted enzyme treatment (Table. 9) and (Figure 19-20). Diverse findings were observed in studies on the effect of seed enzyme pre-treatment on extracted oil phenolic components. Pretreatment of borage seeds with an equal mixture of Olivex and Celluclast at 0.3% enzyme to substrate ratio, 45°C for 9 hours, greatly increased the total phenolic compounds in the methanol oil extracts from 76.71 to 123.42 g Catechin/kg<sup>[41]</sup>. When oilseeds are roasted, phenolic compounds are released from their bounded state and undergo a chemical transition that occurs because of the heat treatment. This allows the phenolic compounds to enter the oil phase. The findings indicate that the microwave-assisted enzyme treatment increased the amount of total phenolic compounds in oil, compared to the seeds described earlier. This rise may be attributed to newly generated or released chemicals as a result of heating and the breakdown of cell walls by enzymes that may be more lipophilic and able to enter oil. Additionally, the work's observation of a deeper oil color at higher roasting temperatures may help to explain the significant production and transfer of novel compounds with potent antioxidant action into the oil.

#### 4. CONCLUSION

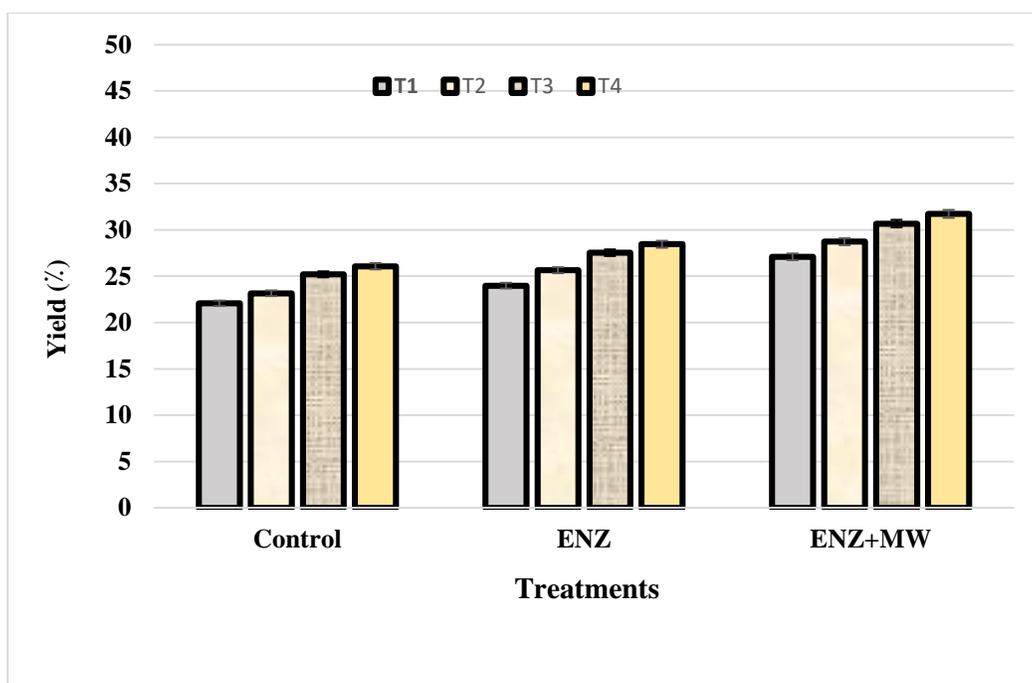
The current investigated the impact of temperature, enzyme treatment, and microwave enzyme treatment on the physicochemical parameters of safflower oil varieties. The study aimed to elucidate how these treatments influenced the quality and characteristics of safflower oil, which holds significant importance in various industrial and culinary applications. Through careful experimentation and analysis, we observed distinct effects of each treatment on the physicochemical parameters of safflower oil such as yield, density, peroxide value, iodine value, saponification value, and antioxidant activity. In comparison to variety B of Safflower, A variety represents more oxidative stability throughout the experimentation. Temperature, enzyme (ENZ), and microwave-assisted enzyme (ENZ+MW) treatment exerted noticeable changes in the oil's properties, with higher temperatures correlating with a decrease in density, iodine value, and saponification value. Microwave treatment, on the other hand, demonstrated the potential to induce modifications in the oil's composition and stability. Notably, the introduction of pectinase enzyme treatment emerged as a promising avenue for enhancing certain aspects of safflower oil. Our findings revealed improvements in emulsification properties and potential reductions in oil density, iodine value, and saponification suggesting the enzymatic action's capacity to modulate the oil's structural attributes. Results of iodine value and saponification value are further supported by FTIR spectroscopy indicating the higher temperature decreases the intensity of band at 3006 cm<sup>-1</sup> indicating the reduction of unsaturation. These results underscored the intricate relationship between processing techniques and the physicochemical characteristics of safflower oil. Furthermore, they highlight the importance of optimizing treatment parameters to achieve desired outcomes

while minimizing undesirable alterations. Overall, our study contributes the valuable insights into the effects of temperature, microwave treatment, and pectinase enzyme treatment on safflower oil's physicochemical parameters. These findings hold implications for industries and researchers seeking to enhance safflower oil quality, functionality, and applicability across various domains. Future research endeavors may delve deeper into mechanistic insights and explore additional treatment modalities to further refine safflower oil processing techniques and maximize its potential utility.

**Table 1: Percentage yield of Safflower Oil Seed**

Temperature	Variety A			Variety B		
	Control	ENZ	ENZ+MW	Control	ENZ	ENZ+MW
T <sub>1</sub>	22.07±0.29	23.98±0.32	27.09±0.36	22.82±0.30	24.73±0.33	27.34±0.36
T <sub>2</sub>	23.15±0.30	25.65±0.34	28.73±0.38	23.90±0.31	28.40±0.37	30.88±0.41
T <sub>3</sub>	25.21±0.33	27.54±0.36	30.68±0.40	25.96±0.34	30.29±0.40	31.73±1.42
T <sub>4</sub>	26.08±0.34	28.46±0.37	31.73±0.42	26.83±0.35	31.21±0.41	33.48±0.44

The values are mean ± SD, each oil sample was analysed individually in triplicate ( $P < 0.05$ ).



**Figure 1: Percentage Yield of Safflower Oil Seed Variety A**

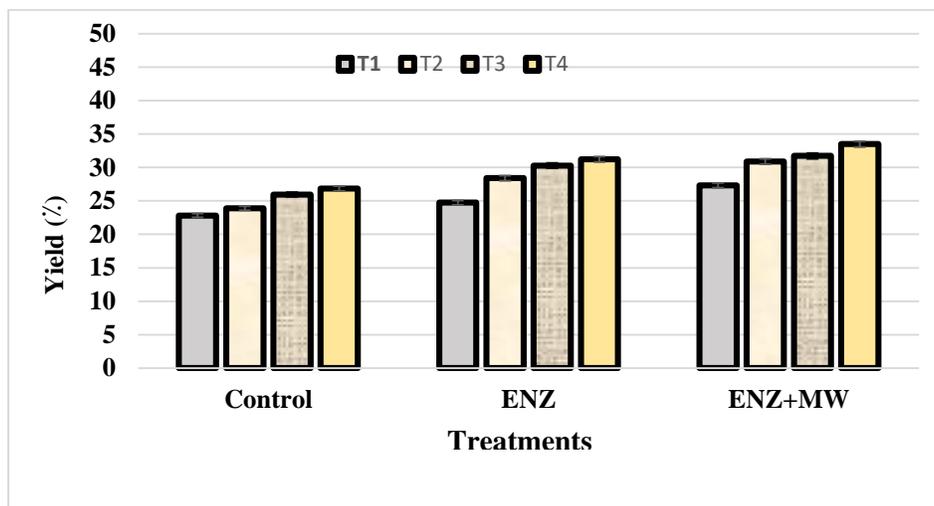


Figure 2: Percentage Yield of Safflower Oil Seed Variety B

Table 2: Density of Safflower Oil Seed Varieties

Temperature	Variety A			Variety B		
	Control	ENZ	ENZ+MW	Control	ENZ	ENZ+MW
T <sub>1</sub>	0.991±0.022	0.976±0.022	0.965±0.022	0.998±0.687	0.981±0.021	0.976±0.023
T <sub>2</sub>	0.567±0.013	0.765±0.017	0.78±0.017	0.660±0.015	0.752±0.017	0.689±0.016
T <sub>3</sub>	0.435±0.10	0.452±0.010	0.654±0.015	0.600±0.014	0.615±0.014	0.492±0.011
T <sub>4</sub>	0.239±0.005	0.342±0.007	0.331±0.007	0.509±0.012	0.332±0.008	0.257±0.006

The values are mean ± SD, each oil sample was analysed individually in triplicate ( $P < 0.05$ ).

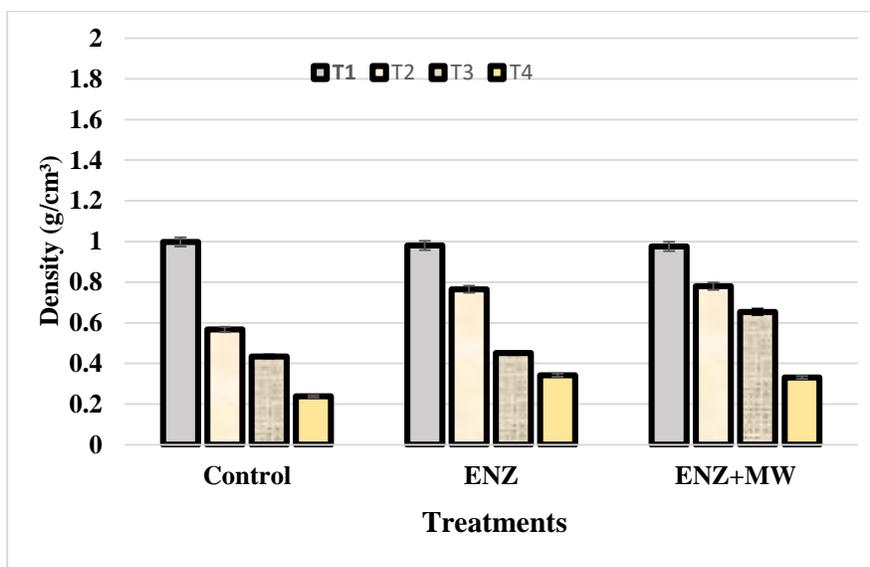


Figure 3: Density of Safflower Oil Seed Variety A

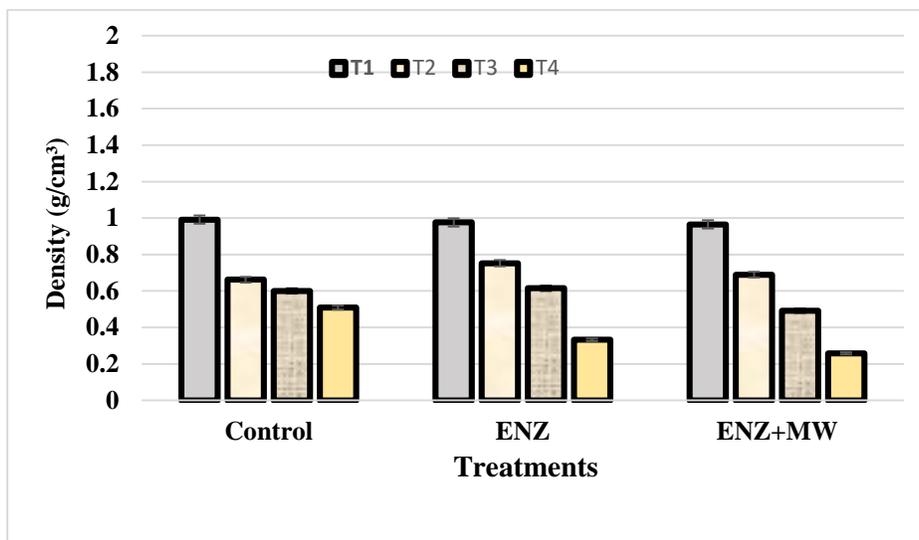


Figure 4: Density of Safflower Oil Seed Variety B

Table 3: Refractive Index of Safflower Oil Seed Varieties

Temperature	Variety A			Variety B		
	Control	ENZ	ENZ+MW	Control	ENZ	ENZ+MW
T <sub>1</sub>	1.469±0.034	1.469±0.034	1.469±0.034	1.472±0.034	1.472±0.034	1.472±0.034
T <sub>2</sub>	1.469±0.034	1.469±0.034	1.469±0.034	1.472±0.034	1.472±0.034	1.472±0.034
T <sub>3</sub>	1.469±0.034	1.469±0.034	1.469±0.034	1.472±0.034	1.472±0.034	1.472±0.034
T <sub>4</sub>	1.469±0.034	1.469±0.034	1.469±0.034	1.472±0.034	1.472±0.034	1.472±0.034

The values are mean ± SD, each oil sample was analysed individually in triplicate ( $P < 0.05$ ).

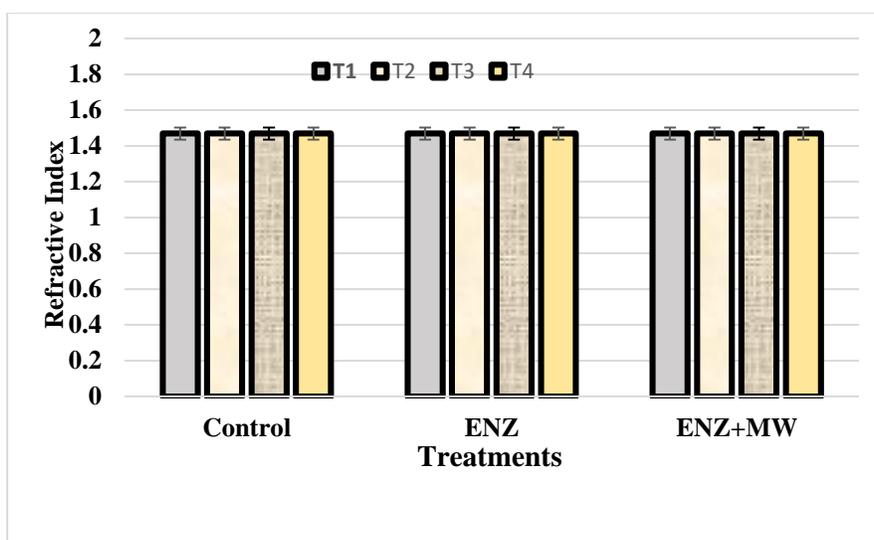
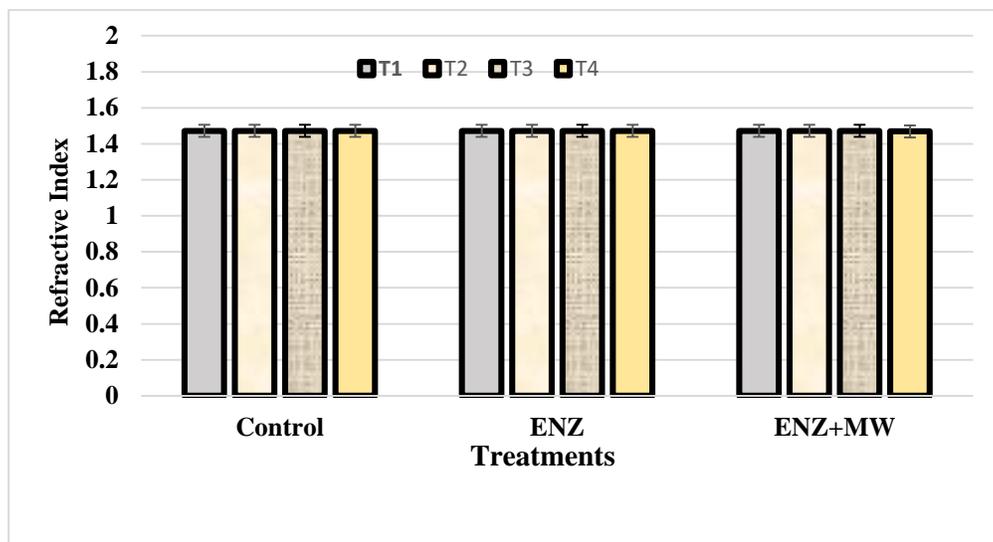


Figure 5: Refractive Index of Safflower Oil Seed Variety A

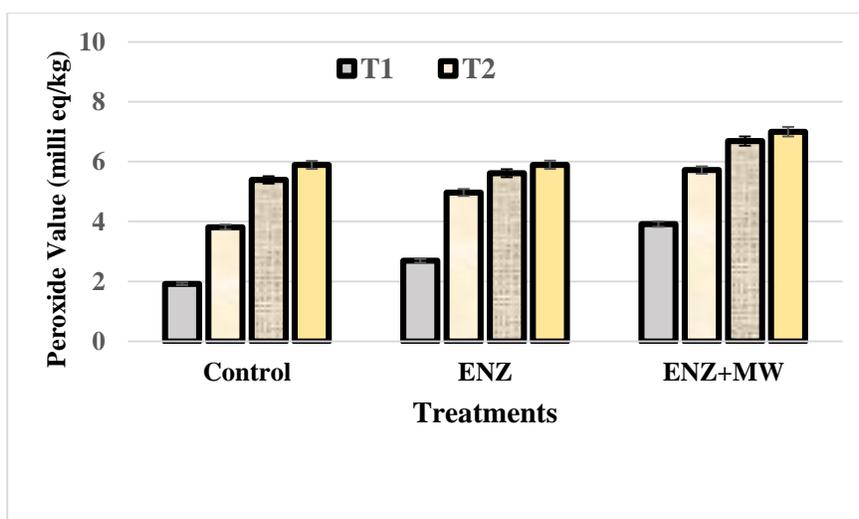


**Figure 6: Refractive Index of Safflower Oil Seed Variety B**

**Table 4: Peroxide Value of Safflower Seed Oil Varieties**

Temperature	Variety A			Variety B		
	Control	ENZ	ENZ+MW	Control	ENZ	ENZ+MW
T <sub>1</sub>	1.922±0.044	2.691±0.062	3.911±0.090	2.122±0.049	2.309±0.053	3.109±0.072
T <sub>2</sub>	3.812±0.088	4.969±0.114	5.723±0.121	3.612±0.083	4.169±0.096	5.323±0.122
T <sub>3</sub>	5.389±0.124	5.615±0.129	6.689±0.154	4.189±0.096	5.367±0.123	6.189±0.142
T <sub>4</sub>	5.888±0.135	5.895±0.136	6.999±0.161	4.888±.112	6.095±0.161	6.999±0.161

The values are mean ± SD, each oil sample was analysed individually in triplicate ( $P < 0.05$ ).



**Figure 7: Peroxide value of Safflower Oil Seed variety A**

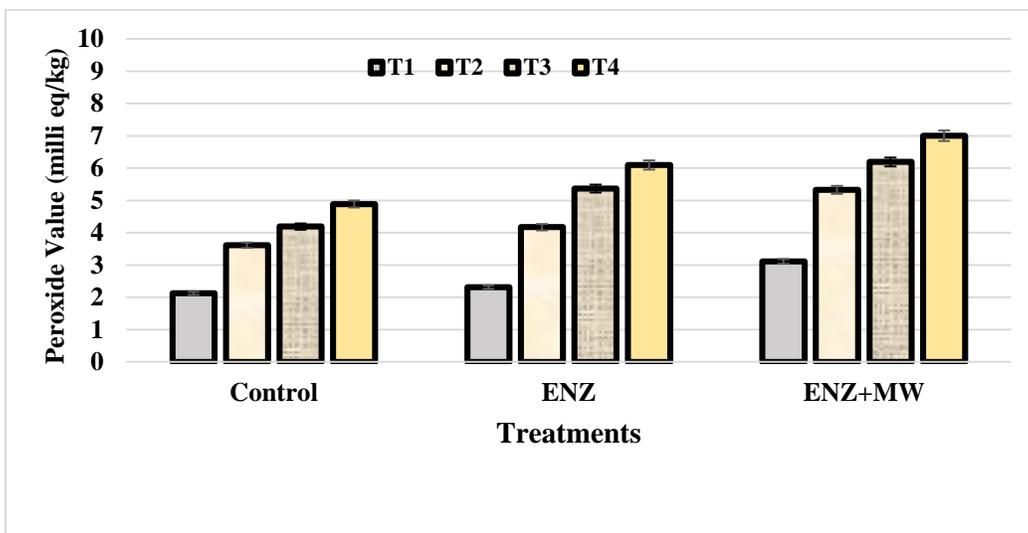


Figure 8: Peroxide Value of Safflower Oil Seed Variety B

Table 5: Iodine Value of Safflower Seed Oil Varieties

Temperature	Variety A			Variety B		
	Control	ENZ	ENZ+MW	Control	ENZ	ENZ+MW
T <sub>1</sub>	126.0±0.828	128.0±0.841	131.0±0.860	128.0±0.841	129.0±0.847	133.0±0.874
T <sub>2</sub>	125.0±0.821	126.0±0.828	129.0±0.847	127.0±0.834	128.0±0.841	131.0±0.860
T <sub>3</sub>	123.0±0.808	125.0±0.821	127.0±0.834	125.0±0.821	126.0±0.828	129.0±0.847
T <sub>4</sub>	122.0±0.801	123.0±0.808	125.0±0.821	122.0±0.801	123.0±0.808	128.0±0.834

The values are mean ± SD, each oil sample was analysed individually in triplicate ( $P < 0.05$ )

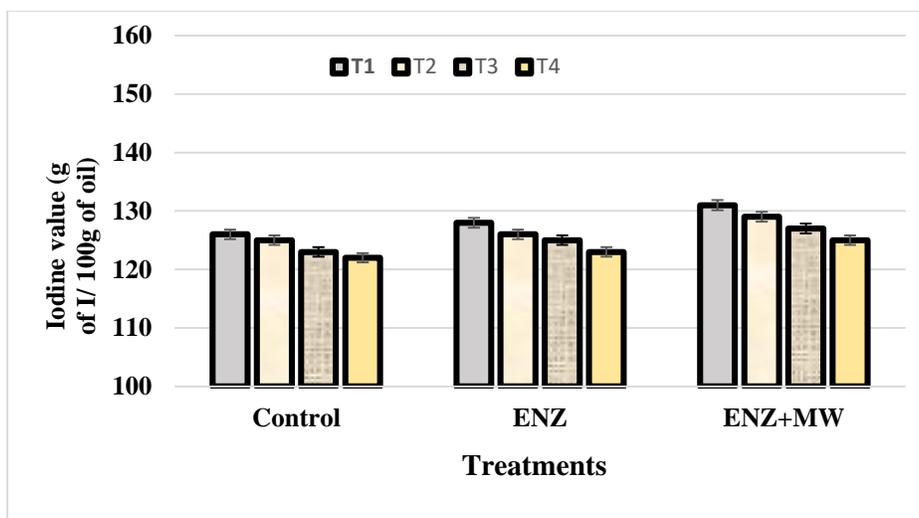


Figure 9: Iodine Value Comparison of Safflower Seed Oil Variety A

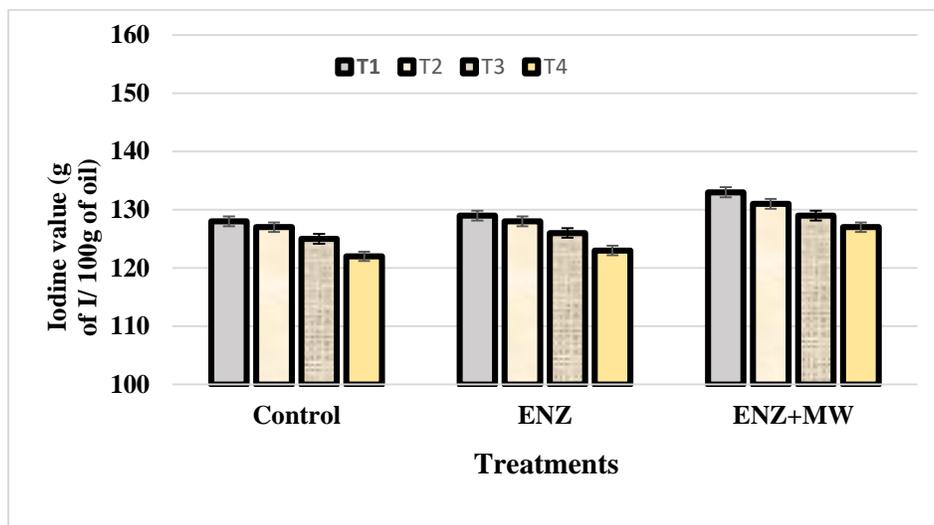


Figure 10: Iodine Value Comparison of Safflower Seed Oil Variety B

Table 6: Saponification Value of Safflower Seed Oil Varieties

Temperature	Variety A			Variety B		
	Control	ENZ	ENZ+MW	Control	ENZ	ENZ+MW
T <sub>1</sub>	199.0±1.045	197.0±1.428	190.0±1.378	202.0±0.866	201.0±0.861	198.0±0.849
T <sub>2</sub>	197.0±1.428	195.0±1.414	186.0±1.349	198.0±0.849	198.0±0.849	196.0±0.840
T <sub>3</sub>	194.0±1.407	190.0±1.378	184.0±1.334	194.0±0.831	195.0±0.836	193.0±0.827
T <sub>4</sub>	190.0±1.378	188.0±1.363	181.0±1.312	192.0±0.823	193.0±0.827	190.0±0.814

The values are mean ± SD, each oil sample was analysed individually in triplicate ( $P < 0.05$ ).

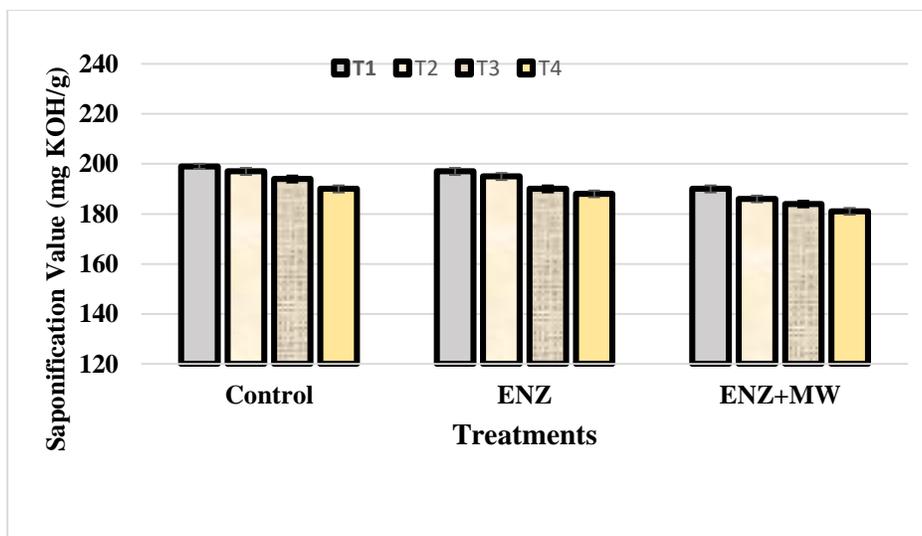


Figure 11: Saponification Value Evaluation of Safflower Seed Oil Variety A

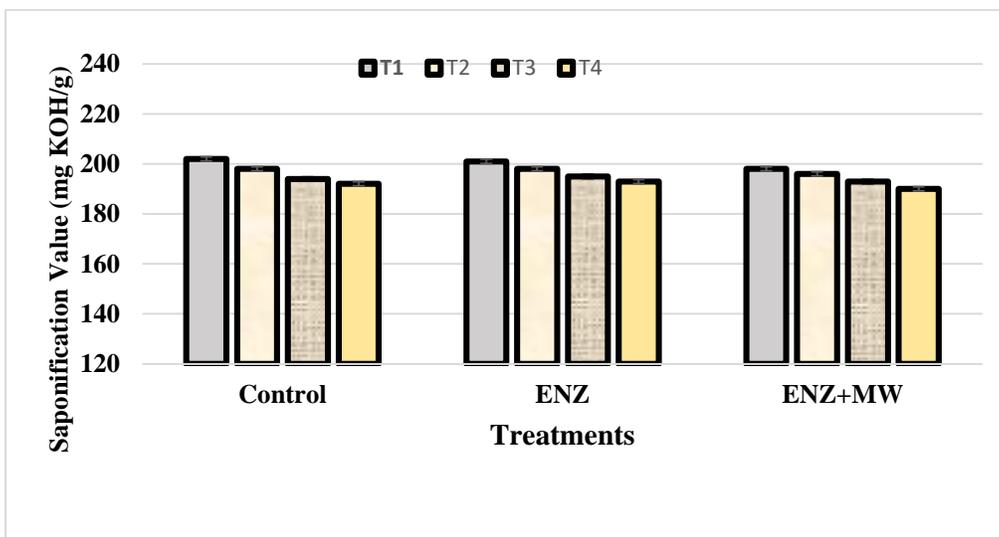


Figure 12: Saponification Value Evaluation of Safflower Seed Oil Variety B

Table 7: Acid Value of Safflower Seed Oil Varieties

Temperature	Variety A			Variety B		
	Control	ENZ	ENZ+MW	Control	ENZ	ENZ+MW
T <sub>1</sub>	0.19±0.001	0.23±0.002	0.16±0.001	0.20±0.001	0.25±0.002	0.19±0.001
T <sub>2</sub>	0.20±0.001	0.25±0.002	0.14±0.001	0.22±0.002	0.27±0.002	0.18±0.001
T <sub>3</sub>	0.21±0.002	0.27±0.002	0.13±0.001	0.23±0.002	0.29±0.002	0.17±0.001
T <sub>4</sub>	0.21±0.002	0.28±0.002	0.11±0.001	0.24±0.002	0.31±0.002	0.16±0.001

The values are mean ± SD, each oil sample was analysed individually in triplicate ( $P < 0.05$ ).

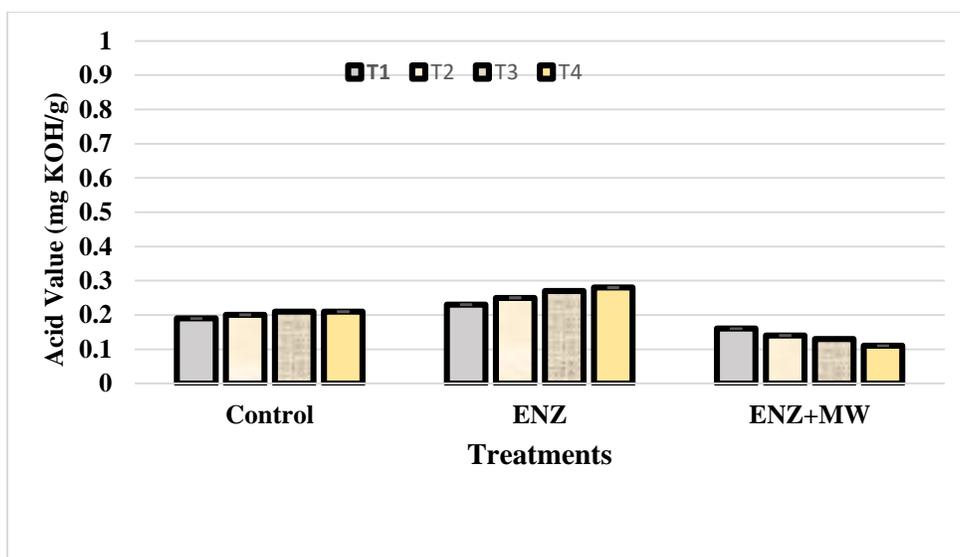


Figure 13: Acid Value Evaluation of Safflower Seed Oil Variety A

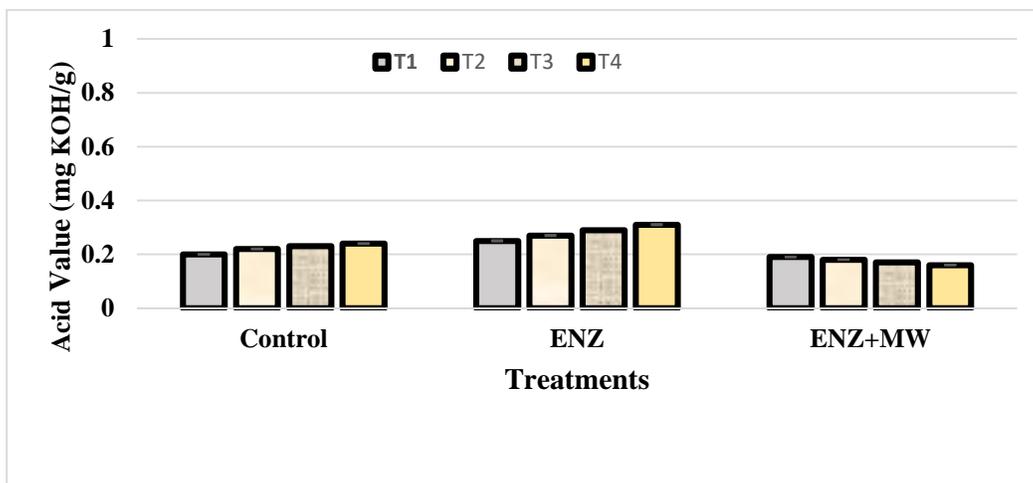


Figure 14: Acid Value Evaluation of Safflower Seed Oil Variety B

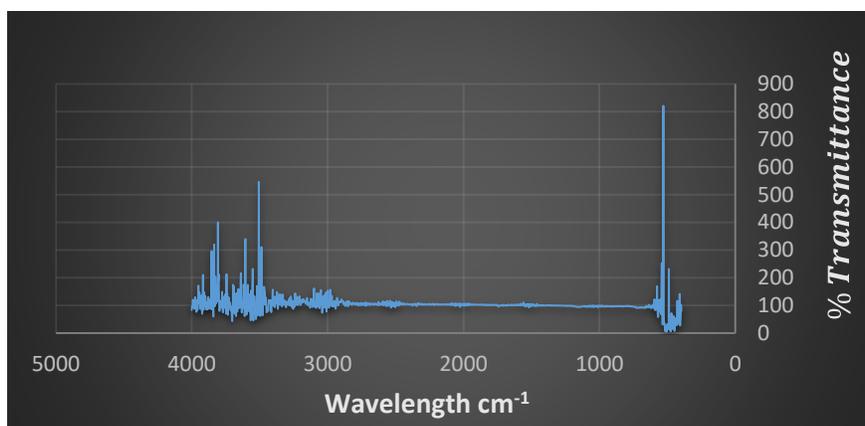


Figure 15a: FTIR Spectra of variety A at 100°C

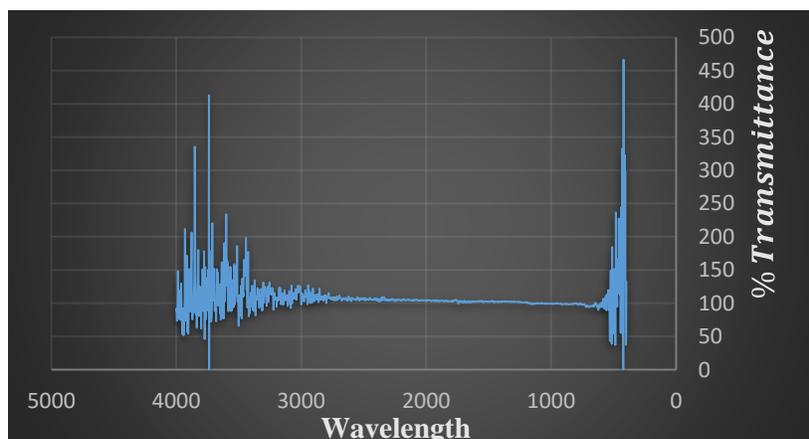
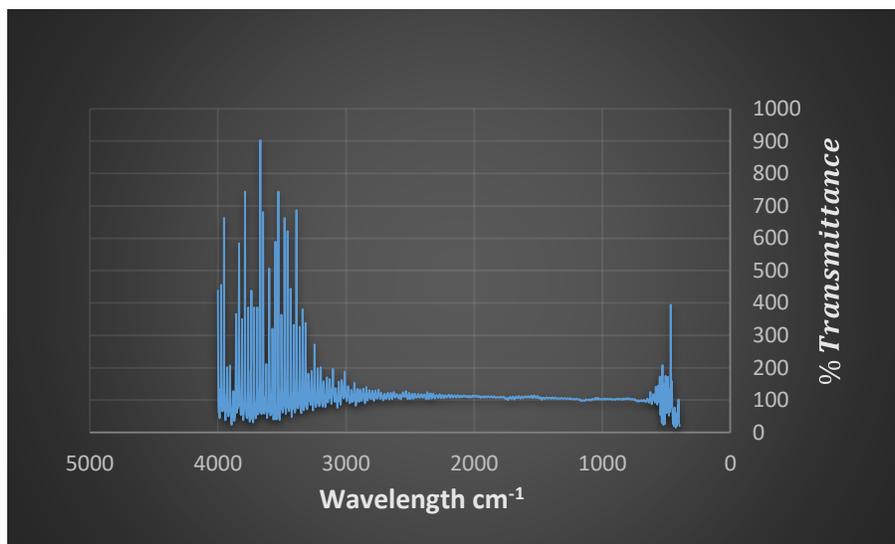
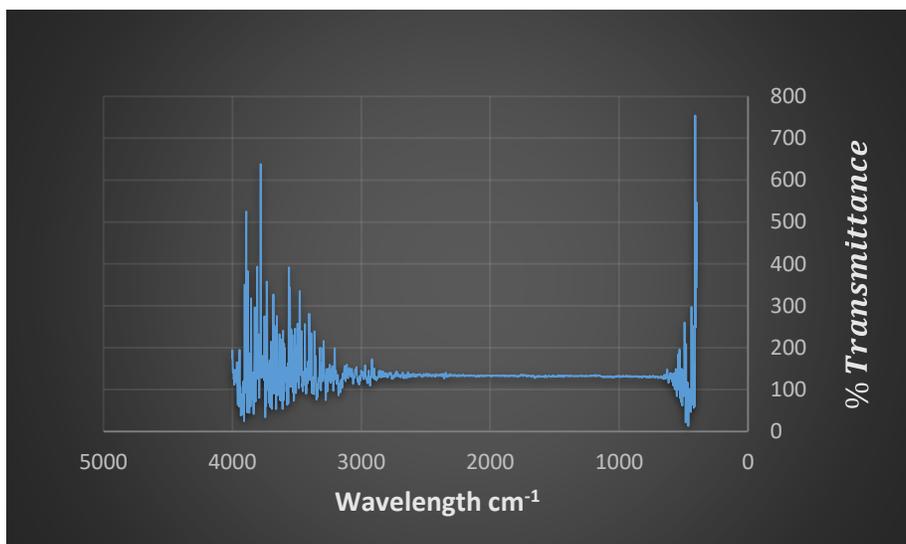


Figure 15b: FTIR Spectra of variety A at 200°C



**Figure 16a: FTIR Spectra of variety B at 100 °C**



**Figure 16b: FTIR spectra of variety B at 200 °C**

**Table 8: DPPH Free Radical Scavenging Assay of Oil Seed Varieties**

Temperature	Variety A			Variety B		
	Control	ENZ	ENZ+MW	Control	ENZ	ENZ+MW
T <sub>1</sub>	0.91±0.009	0.86±0.006	0.81±0.006	1.32±0.009	1.29±0.009	1.17±0.008
T <sub>2</sub>	0.87±0.006	0.73±0.005	0.68±0.005	1.02±0.007	1.11±0.008	0.98±0.007
T <sub>3</sub>	0.71±0.005	0.69±0.005	0.63±0.004	1.00±0.007	0.98±0.007	0.78±0.006
T <sub>4</sub>	0.60±0.004	0.59±0.004	0.57±0.004	0.91±0.006	0.87±0.006	0.68±0.005

The values are mean  $\pm$  SD, each oil sample was analysed individually in triplicate ( $P < 0.05$ ).

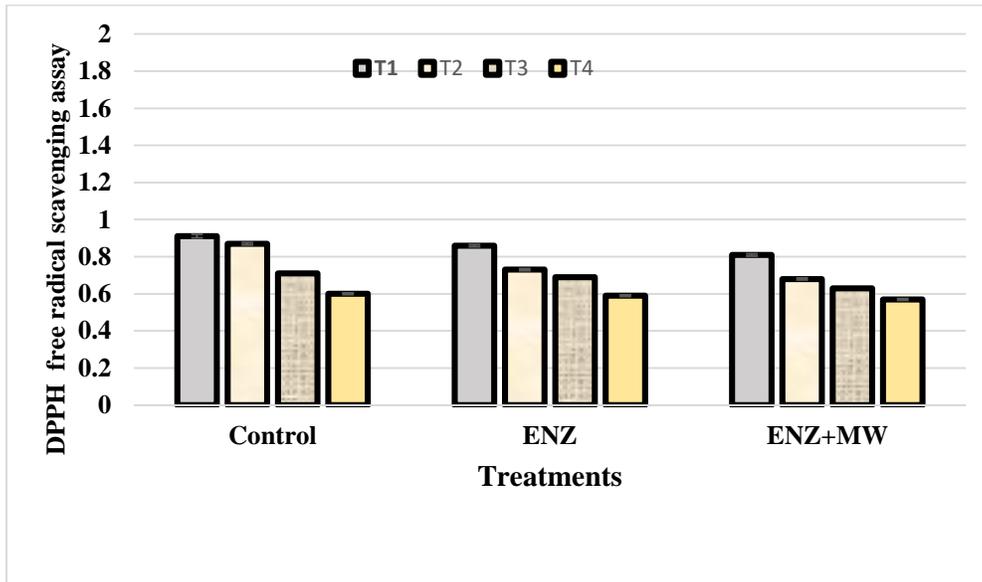


Figure 17: DPPH Free Radical Scavenging Assay of Variety A

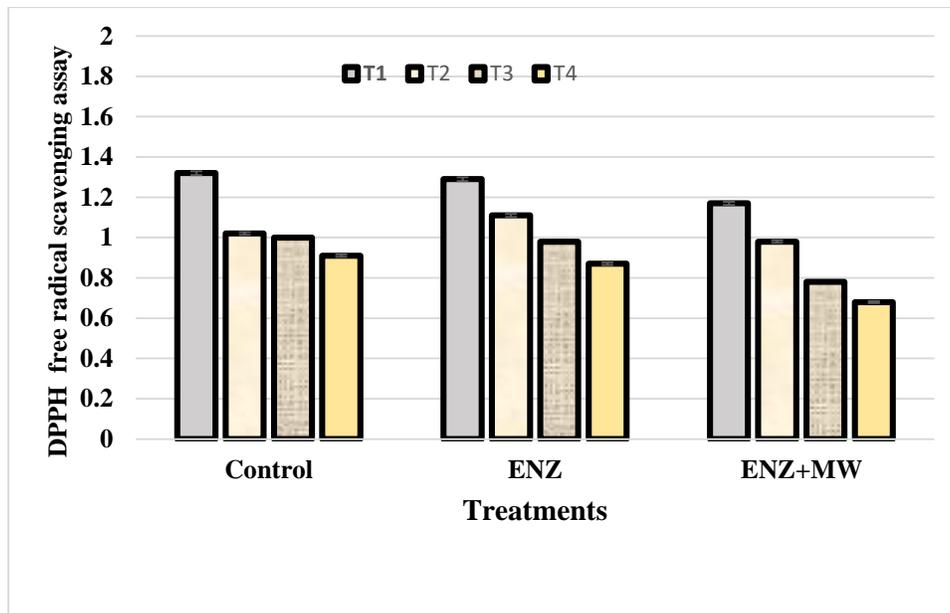
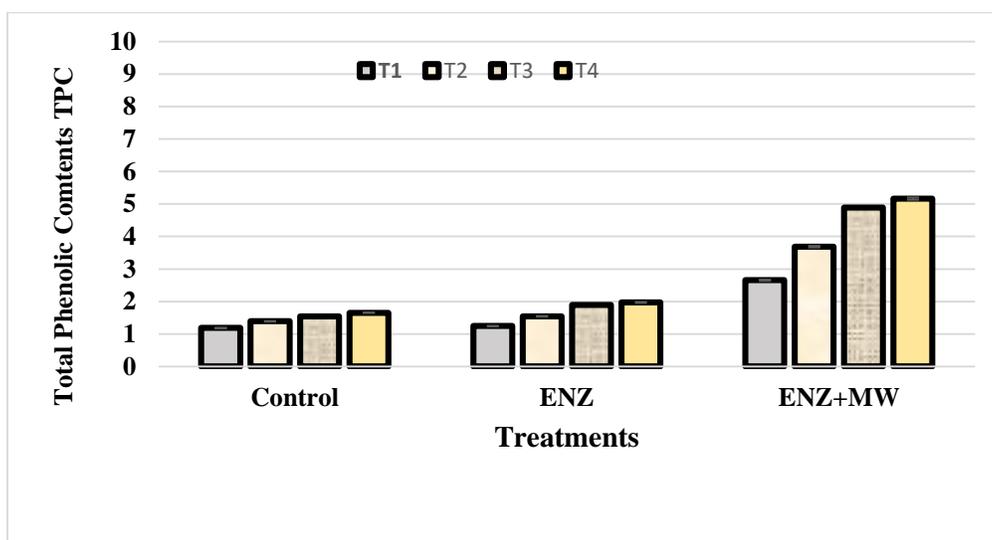


Figure 18: DPPH Free Radical Scavenging Assay of Variety B

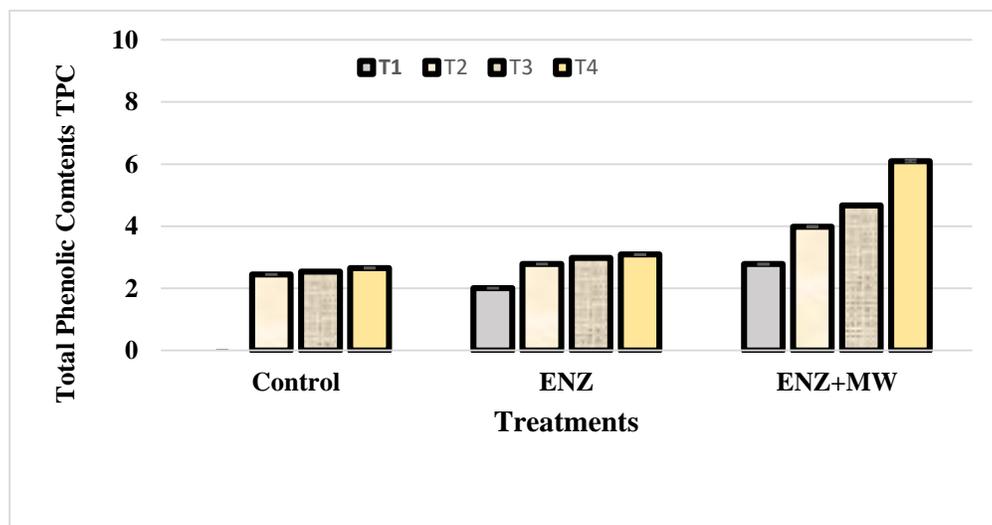
**Table 9: Total phenolic contents (TPC) of Safflower Seed Oil Varieties**

Temperature	Variety A			Variety B		
	Control	ENZ	ENZ+MW	Control	ENZ	ENZ+MW
T <sub>1</sub>	1.185±0.008	1.245±0.009	2.654±0.019	1.985±0.014	2.010±0.014	2.783±0.020
T <sub>2</sub>	1.392±0.010	1.543±0.011	3.689±0.026	2.450±0.017	2.786±0.020	3.986±0.028
T <sub>3</sub>	1.343±0.011	1.890±0.013	4.893±0.035	2.543±0.018	2.983±0.021	4.673±0.033
T <sub>4</sub>	1.654±0.012	1.976±0.014	5.164±0.037	2.654±0.019	3.090±0.022	6.098±0.043

The values are mean ± SD, each oil sample was analysed individually in triplicate ( $P < 0.05$ ).



**Figure 19: Total Phenolic Content of Variety A**



**Figure 20: Total Phenolic Content of Variety B**

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