Research Article

Comparison of the Physicochemical Characteristics of Soybean, Palm, and Hemp Oils as Well as Their Oxidative Stability, and the Impact of Oil Diet on Rat Blood Parameters

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Abstract | The abnormalities of blood lipid levels and cardiac illnesses are clearly related; thus, it is crucial to pay attention to this aspect. Since they include a large proportion of single bond fats, vegetable oils (soybean, hemp, and palm olein) because a reduction in blood lipid levels after meals. Moreover, the level of HDL rises after three weeks of using this oil. The purpose of this study was to examine the fatty acid profile, oxidative stability, and their effect on hematological and cholesterol tests in the rat diet of soybean, hemp, and palm olein. In this study, oxidative stability and the peroxide and iodine index were done as chemical tests, and the components of each were then identified and measured using a gas chromatography-mass spectrometer. Rats were separated into 5 groups of 8 and given treatments of hemp, palm, and soybean totaling 1.5% of their body weight in order to study the effects of various treatments on their blood parameters. Blood tests were used to investigate these parameters. Based on the results, all three oils-hemp, soybean, and palm-had the same refractive index (1.46) and showed no statistically significant different ($P<0.05$). Palm oil had a saponification index of 202 mg/g and a peroxide time of 5.8 hours compared to hemp and soybean oil. The greatest ionic index was found in hemp oil, which had a value of 136. In terms of oxidative resistance, soybean oil was more stable than palm and hemp oils, with a value of 5.25 hours. Linoleic acid (53.8), linoleic acid (57.59), and oleic acid (40/33) are the principal components in soybean oil, hemp oil, and palm oil, respectively. In investigating the effect of consumption, palm, hemp, and soybean oils lowered cholesterol and HDL, but the quantity of blood platelets increased in comparison to the control sample and taking the permitted limit into account. This quantity exceeds the permissible limits in the soybean oil sample (513). The hemp oil sample was taken into account since it had the most unsaturated fatty acids among the quality factors. Palm oil was also considered to be suitable for frying since it had the best oxidative resistance. Hemp oil was suggested because it had indicators of the permitted limit compared to the control sample. This was based on the influence of the oil samples during the period of consumption in the diet of rats.

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1. Introduction

Oil seeds have been utilized for generations to meet medical, food, and heating needs. The use of seeds for oil extraction and the use of protein in human and animal food was recommended as the oil extraction industry expanded. Oil seeds are now regarded as one of the world's strategic agricultural products. According to Iranian Nutrition Institute, oil consumption accounts for 21% of total energy consumption in our country (Izuddin *et al*., 2022). Fats are one of the most significant types of human food, accounting for around 30% of the calories required by the body in a balanced diet. Vegetable and animal fats account for around one-third and two-thirds of total fat consumption, respectively. The types of fatty acids and cholesterol found in vegetable and animal fats differ (Khatun *et al*., 2018). Oleic, linoleic, and linolenic acids, which have one, two, and three double bonds, are the most prevalent unsaturated fatty acids found in association with cholesterol in vegetable oils, and each of them has 18 carbon atoms (Zimborán *et al*., 2022).

One of the most essential characteristics for determining the quality of oils and fats is their oxidation stability. Oil and fat oxidation in air oxygen is an exothermic process that follows the first order of the reaction; hence, thermal analytical techniques are utilized for qualitative analysis (Jadhav *et al*., 2022). Cereals and oil seeds, such as corn, soy, and sesame oil, are the main food sources of fatty acids with many double bonds, such as linoleic acid and linolenic acid (Ahonen *et al*., 2022). Soybean oil contains a variety of fatty acid glycerides, including oleic (43%), linoleic (43%), palmitic (9%), and stearic (4%). Palm oil can be used to enhance the oxidative stability of other edible oils that have a high concentration of unsaturated fatty acids. Palm oil is an edible oil produced from the pulp (meaty component) of the oil palm tree's fruit, and its widespread production in the last three decades has boosted its usage in the fat and oil sector (Halilu *et al*., 2023). Palm oil was formerly exported in its raw form, but it is currently shipped in processed or semi-processed forms, which are palm olein (liquid part) and palm stearin (solid part) (Gunstone, 2018). Ejiofer *et al*. (2021) investigated crude soybean oil qualitative characteristics. The statistical study revealed that free fatty acids ranged from 0.28 to 0.29 percent. The peroxide index ranged from 1.77 to 1.88 meq/kg, whereas the saponification index ranged from 194.12 to 194.88.

Hemp seed contains 20–25% protein, 20–30% carbohydrates, 25–35% oil, and 10-15% insoluble fiber. Edstein, a protein comparable to egg albumin that is easily digestible, is its major protein. Eight important amino acids are found in hemp seeds. Hemp also has a small quantity of digestible carbohydrates, B, C, E, and D vitamins, as well as protein and lipids (Esmaeilzadeh and Dehghan, 2020). Both soluble and insoluble fibers are present in high quantities in hemp seeds, but the insoluble fibers dominate. The majority of these fibers are found in the Hemp shell, which is removed from the seed during the preparation. Peeled hemp provides a minor quantity of dietary fiber (6g/100g) (Asadi *et al*., 2019). The issue of short-chain fatty acids that lead to smoke is not present in palm oil, making it a highly ideal frying oil. There are extremely few fatty acids with polyunsaturated bonds in the oil, which causes oxidation and gives the oil a bad taste and smell. It also has a low iodine index. Palm oil is frequently used as frying oil, either by itself or occasionally combined with soybean oil. The molecular fractionation process separates the liquid part of palm oil, known as palm olein, which contains less saturated fatty acid and fatty solids than palm (Halilu *et al.*, 2023). Since it contains a high proportion of carotenoids and around 50% of the palmitic fatty acid, palm oil has high oxidative stability. The presence of high saturated fatty acids, however, is a known risk factor for a number of diseases, including atherosclerosis and cardiovascular disease (Andrade *et al.*, 2022).

One of the leading causes of mortality in adults is cardiovascular disease, particularly during the fourth decade of life. One of the four major risk factors for this ailment is high blood lipids, which may be controlled by nutrition. Cholesterol can raise the risk of cardiovascular illnesses even though it is involved in many crucial metabolic processes in the body. Some cholesterol oxidation byproducts are also referred to as mutagens and carcinogens (Sadeghi *et al.*, 2021). Another risk factors for cardiovascular illnesses are hypercholesterolemia. The general health of the body is impacted by a rise in blood cholesterol, which also raises the mortality rate from cardiovascular illnesses. Hypercholesterolemia is more common in industrial societies, and the major reason is poor diet, which comprises foods high in saturated fat and cholesterol. Given that lecithin lowers cholesterol and that cardiovascular diseases brought on by hypercholesterolemia are prevalent in our country, more investigation on this subject is obviously required (Khani *et al.*, 2019). According to Jalali *et al.* (2018), rainbow trout's growth responses and blood biochemical parameters can be affected when fish oil is substituted with soybean or rapeseed oil in the diet. In addition, fish blood triglycerides and cholesterol both significantly increased as a result of soybean oil consumption. The intake of vegetable oil after three weeks significantly raises the quantity of HDL (Suman *et al.*, 2023). Many phenolic compounds, including flavonoids like lutein and apigenin, phenolic acids, and phenyl alcohols such as tyrosol, hydroxytyrosol, are found in soybean oil. Vegetable oils are becoming more popular due to their advantages for human health. Since it contains a lot of oleic acid, a kind of monounsaturated fatty acid, it lowers blood fat levels as well as the quantity of low-molecular-weight lipoprotein in the blood after a meal (Dehghan *et al.*, 2022). In this study, the fatty acid composition and oxidative stability of soybean, hemp, and palm olein as well as their impact on cholesterol and hematological tests in the diet of rats were examined in order to establish the function and significance of these oils.

2. Materials and Methods

2.1 Materials

At the Farahada Oil Company and Research Institute in Kashmar City, refined soybean, hemp, and palm oils were made with no antioxidants and stored at -4 degrees Celsius in the dark until the tests were conducted. All chemicals and solvents were acquired from Merck in Germany. Devices include: Rancimat device (Switzerland, Herisau, Metrohm Ltd), Gas Chromatography device (South Korea, Anyang, Young Lin Bldg.), electric heater (Germany Gerhardt-RC-50), Refractometer (ATAGO A2207-Japan), Bain Marie (Memmert wb14-Germany), Mini spin plus micro centrifuge machine (14500 rpm), (Eppendorf AG, Germany), Leitz microtome model 1512, Germany, Normal microscope model Olympus CH2 Japan, graduated eyepiece or graticule, Kits for measuring biochemical factors from Pars Azmoun company.

2.2 Methods

Two tests were conducted as part of this study at the Kashmar branch of the Islamic Azad University. Regarding oxidative stability, fatty acid profile, and physicochemical examinations, palm, soybean, and hemp oils were compared in the first stage under the same purifying procedures. The second stage involved examining how three different kinds of oil affected the blood parameters of rats.

2.2.1 Rancimat test

The Rancimat equipment was used to test 2.5 grams of the sample that was subjected to air flowing continuously at a rate of 20 liters per minute at a temperature of 120 degrees Celsius (Elhami *et al.*, 2019).

2.2.2 Determination of peroxide value

AOAC-21.1.41 technique was used to measure iodometric titration. A 30 cc solution of chloroform acetic acid was combined with one gram of the oil sample. It was then mixed with 20 cc of distilled water and 0.5 cc of saturated KI solution (because the titration reaction requires an aqueous medium). As soon as the iodine was released, it was titrated until the desired lemon yellow color was achieved with 0.1

normal sodium thiosulfate. The following equation was used to compute the peroxide index (Adryu *et al.*, 2019). In this equation, V1 and V2 represent the volumes of thiosulfate used for the control and sample, respectively. N refers to the normality of the thiosulfate used, and M stands for the weight of the oil used per gram.

$$Peroxide\ index = \frac{V_1 - V_2 \times N \times 1000}{M} \quad \ldots (1)$$

2.2.3 Iodine index

The solution was colored salmon with the addition of 0.5 g of the sample, 10 ml of chloroform as a solvent, and 25 ml of Hatos reagent. After that, Erlene was left in the dark for 30 minutes to complete the addition reaction. The sample was then mixed with 15 ml of a 15% potassium iodide solution, 10 ml of distilled water, and 1 ml of starch glue. Titration was then performed using 0.1 normal sodium thiosulfate and continued until a lemon yellow color emerged (Gordon and Mursi, 2017).

Iodine index=1.269×Normality of sodium thiosulfate (the volume of thiosulfate consumed by the sample - the volume of thiosulfate consumed by the control) …(2)

2.2.4 Saponification index

The flask was filled with 50 ml of potassium hydroxide and 5 grams of oil sample before being connected to the refrigerant and set on the heater. A half-hour of heating was completed. A few drops of the phenolphthalein reagent were added to the flask when it had completely cooled, and the pink tint was gradually eliminated by titrating the solution with 0.5 normal hydrogen chloride. The following equation was utilized to determine the saponification index (AOAC, 2015).

Saponification index= molecular weight of potash x (volume of acid used in the sample - volume of acid used in the control)/ weight of the sample …(3)

2.2.5 Fatty acid composition

The AOAC (1993) method was used to compile the fatty acid profile. Hence, first, fatty acid methyl ester was prepared and its composition was identified. The fatty acid composition of oils was evaluated using a gas chromatography (GC-17A), a capillary glass column (length 30 meters filled with diethylene glycol succinate, inner diameter 0.22 mm), and a flame ion detector (FID). This was accomplished by dissolving around 0.3 grams of test oil in 7 ml of n-hexane, adding 2 ml of normal methanolic potassium hydroxide solution, and mixing at 50 degrees Celsius. The solution was then injected with a split needle into 0.4 ml at 150 °C (with a ratio of 20:1). The sample injection site, the column, the detector, and the flow rate of the carrier gas (nitrogen) were all at 230°C, 200°C, 250°C, and 10 mm/min, respectively (Ashuri *et al.*, 2021).

2.2.6 Refractive index

This test was carried out in accordance with Iranian national standard No. 5108. In this experiment, the sample's refractive index was measured using a refractometer at a temperature of 20 degrees celsius (Farhoosh *et al.*, 2018). Equation 4 is used to determine the refractive index "nDt" when the difference between the measurement temperature "t1" and the reference temperature "t" is less than 3°C:

T_1 is the measurement temperature in Celsius, T is the reference temperature in Celsius.

$$n_D^t = n_D^{t_1} + (t_1 - t)F \quad \ldots (4)$$

2.3 The second part of the research: investigating the effect of different levels of hemp oil, soybean oil and palm oil on blood parameters in rats

2.3.1 Statistical population

40 male Wistar rats weighing 200–250 grams each made up the study's population. They were housed in the Mashhad Research Institute's animal house, where they were subjected to 12-hour cycles of light and darkness, 23°C temperatures, and air humidity levels of 40–60%. Both food and water were available to the animals under investigation.

Rats were divided into 30 groups, each receiving 5 treatments. Each treatment included 6 rats and a completely random model. They were given food and drink every day.

- The first group: Rats used as controls did not consume any soybean, hemp, or palm oils.
- The second group: rats given hemp oil, which made for 1.5% of their body weight.
- The third group: rats given palm oil, which made for 1.5% of their body weight.
- The forth group: rats given soybean oil, which made for 1.5% of their body weight.

The rats were anesthetized using an ether solution after the 15-days treatment period was complete, and blood was drawn straight from the heart of the animals using a syringe dipped in heparin. The serum was separated from the blood using a 1700 rpm centrifuge for 15 minutes after the blood was poured into 1.5 cc vials. Platelets were measured and analyzed using specialized laboratory kits and an auto analyzer (model BT-3500) in the pathology laboratory in Kashmar city, along with other blood parameters as HDW, triglyceride, cholesterol, and HDL.

2.4 Statistical analysis

The experiments were run three times, and the data was analyzed using the random-factorial experimental design and SPSS version 26 software (SPSS 26.0 for Windows, SPSS Inc., Chicago, IL, USA). The Duncan's multiple range test ($P < 0.05$) was used to evaluate whether there was a significant difference between the means.

3. Results and Discussion

3.1 Investigating the physicochemical properties of palm oil, soybean oil, and hemp oil

Figure 1 shows the variance analysis of the oxidative stability indexes of hemp oil, palm oil, and soybean oil. The results indicated that oil at 130 degrees celsius had a significant, 5%-level impact on the oxidation stability index. Hemp oil, soybean oil, and palm oil were revealed to have oxidation stability indexes of 2.695, 5.250, and 4.170 hours, respectively.

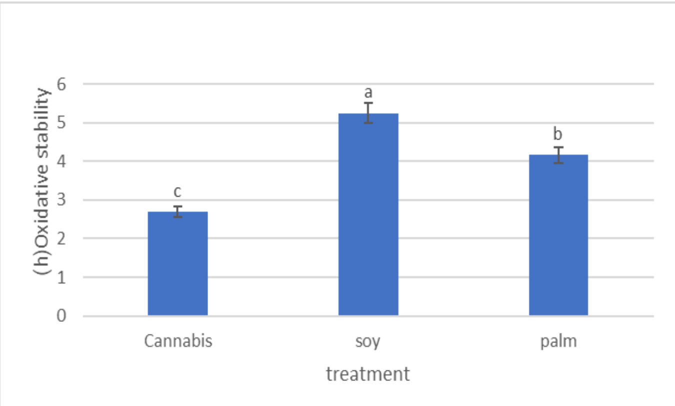

Figure 1: The effect of oil type on the oxidation stability index (OSI).

In accordance with variance analysis, the soybean oil sample had the highest level of oxidative stability and differed statistically significantly from the palm and hemp treatments ($p<0.05$).

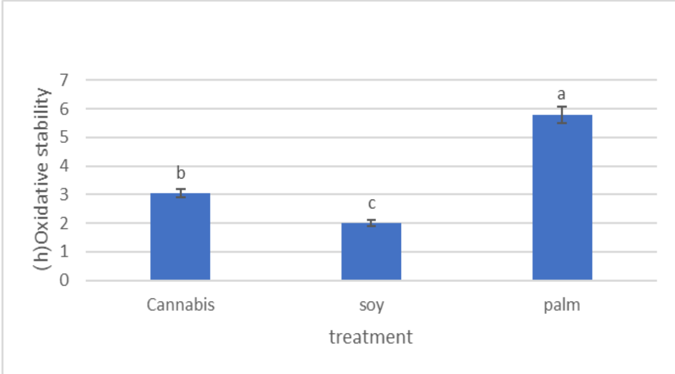

Figure 2: Effect of oil type on peroxide index.

Figure 2 shows the variance analysis of the peroxide of hemp oil, soybean oil, and palm oil. The findings demonstrated that peroxide was significantly affected by oil type at the 5% level. So, the peroxide value of soybean oil was 2.01 meq/kg, 3.05 meq/kg for hemp oil, and 5.8 meq/kg for palm oil.

Table 1: The average composition of fatty acids in palm oil, hemp and soybean oil.

Type of fatty acid	Palm oil	Hemp oil	Soybean oil
Palmitic acid	37.94^{A}	6.625^{C}	11.625^{B}
Palmitoleic acid	0.128^{A}	0.13^{A}	0.091^{B}
Heptadesenoic acid	0.081^{B}	0.13^{A}	0.1^{B}
Margaric acid	0.097^{C}	0.16^{A}	0.1115^{B}
Stearic acid	4.49^{A}	2.58^{B}	4.425^{A}
Oleic acid	40.33^{A}	17.16^{C}	22.4^{B}
Linoleic acid	15.72^{C}	57.59^{A}	53.8^{B}
Linolenic acid	3.64^{C}	15.31^{A}	7.817^{B}
Arachidonic acid	0.132^{C}	0.31^{B}	0.6^{A}
SFA saturated fatty acids	42.43^{A}	19.66^{B}	17.17^{C}
Mono and polyunsaturated	40.33^{C}	90.34^{A}	83.35^{B}
cis fatty acids	49.47^{C}	90.31^{A}	83.28^{B}
other	1.71^{A}	0.1^{C}	0.15^{B}

Figure 2 shows that the effect of oil type on peroxide was significant at the 5% level, including the largest quantity identified in the palm oil sample ($p<0.05$). Table 1 provides the fatty acid profiles of soybean, palm, and hemp oil samples. The analysis of the average fatty acid composition of palm, hemp, and soybean oils revealed that palm oil had the largest proportion of saturated fatty acids, with 42.43 percent of saturated fatty acids overall and 37.9 percent of palmitic acid. Hemp oil and soybean oil's primary fatty acids, linoleic acid, were found to be 57.59 and 53.8, respectively. Palm oil had the most, 33.40, oleic acid. Saturated fatty acids made up 19.66 of the hemp oil's composition, while unsaturated fatty acids made up 90.31. Oleic acid had the maximum number of fatty acids, whereas heptadesenoic acid and palmitoleic acid had the lowest amounts.

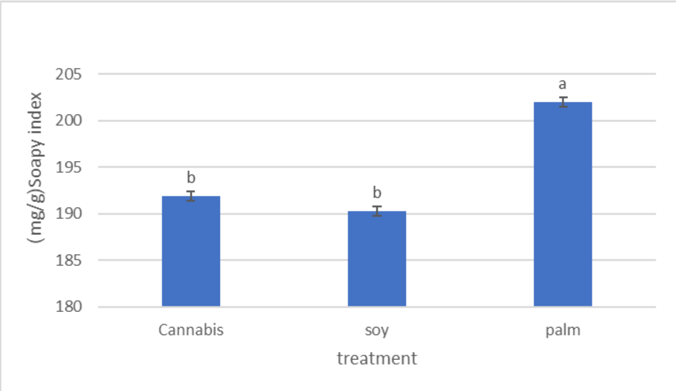

Figure 3: Comparison of saponification index in palm, soy, and hemp oil samples.

Figure 3 presents the variance analysis of the saponification indexes of hemp oil, palm oil, and soybean oil. The findings demonstrated that the saponification index was significantly influenced by oil type at a level of 5%. Hemp oil, soybean oil, and palm oil all had saponification indexes of 191.9 mg/g, 190.25 mg/g, and 202 mg/g, respectively.

The analysis of the saponification index revealed that the kind of oil significantly influenced the saponification index's value ($p<0.05$). The palm oil treatment had the greatest numerical value, but there was no statistically significant difference between the soybean and hemp oil treatments ($p>0.05$).

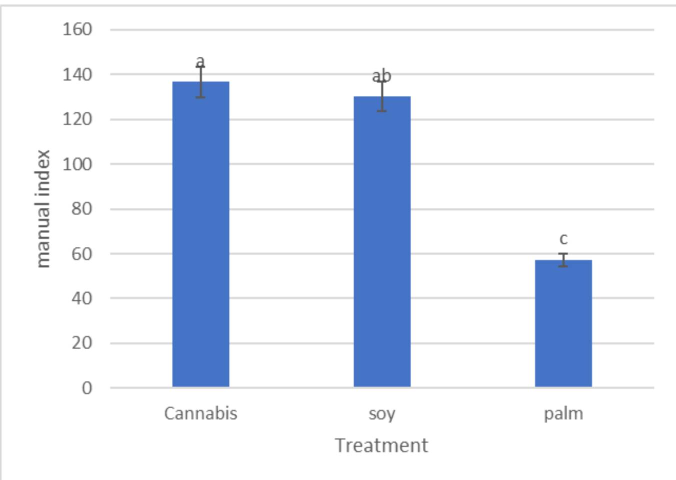

Figure 4: The effect of oil type on the iodine index in oil samples.

In Figure 4, the variance analysis of the iodine index of hemp, soybean, and palm oil are presented. Compared to soybean and palm oil, hemp oil has a higher iodine index. The iodine indexes for soybean and palm oils were 130/25 and 57/30, respectively, whereas hemp oil had a value of 136/87.

Figure 4 displays the iodine index's findings ($p<0.05$). The maximum quantity was recorded in the hemp treatment, which did not differ significantly from the soy oil treatment, but there was a significant difference at the 5% level in the palm oil treatment ($p<0.05$).

The findings demonstrated that there was no significant difference in the refractive indices of the three samples of soybean, palm, and hemp oil at the probability level of 0.05; these values were 1.467, 1.458, and 1.467, respectively.

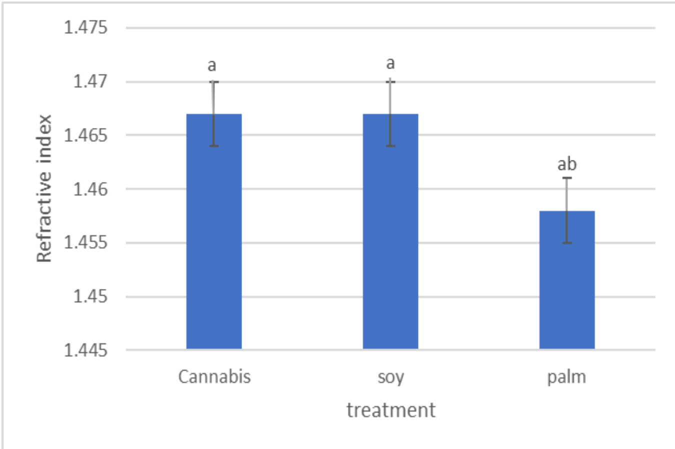

Figure 5: Effect of oil type on refractive index.

Figure 5 demonstrated that the refractive index was significantly influenced by the kind of oil ($p<0.05$). There was significant variation with the palm oil treatment ($p>0.05$), but not with the hemp or soy oil treatments ($p<0.05$). The palm oil treatment was found to have the lowest value.

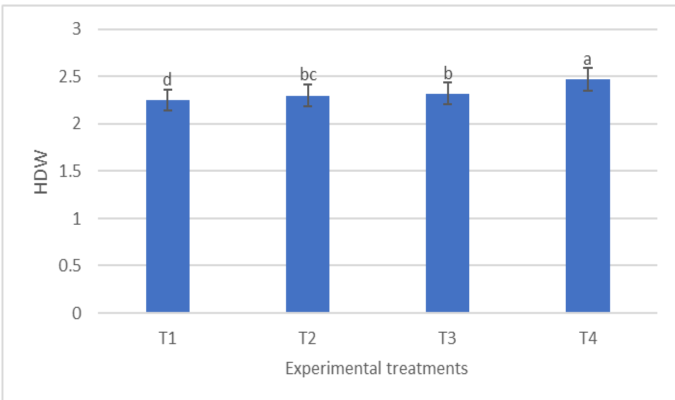

Figure 6: Effect of diet containing different oils on HDW factor in rats.

Table 2: The effect of the use of oils in the diet of rats on blood parameters.

Blood factor	Control blood sample (T_1)	Blood sample containing hemp oil (T_2)	Blood sample containing soybean oil (T_3)	Blood sample containing palm oil (T_4)	P value	SEM
Cholesterol	84	75.5	80	93	0.010	0.273
HDL	34.5	32.5	32	32	0.033	0.140
Tri glyceride	122	78	93	47	0.065	0.287
Platelet	437.5	457.7	513	450.5	0.044	1.148

3.2 The effect of different levels of hemp, soybean, and palm oil on blood parameters in rats

Figure 6 depicts the effects of various treatments on the distribution range of hemoglobin in samples of soybean, palm, hemp, and the control sample ($P<0.05$). The samples subjected to the palm oil diet showed the highest changes in this factor. The highest quantity of HDW was found in the palm oil treatment (2.4), followed by hemp oil and soybean oil (2.3 and 2.32, respectively). This value was 2.25 in the control sample, which was significantly different from what was observed in the other samples ($P<0.05$).

Figure 6 displayed the effects of several treatments on the hemoglobin distribution range for soybean, palm, hemp, and the control sample. According to the findings, the HDW factor was significantly affected by the diets containing various oils ($P<0.05$), with the palm oil diet samples showing the greatest change in this factor.

The treatments significantly affected blood cholesterol levels, according to the analysis of variance ($P<0.05$). Table 2 shows that the treatment containing palm oil had the greatest level of cholesterol, followed by the control, which had 93 and 84, respectively. It is evident from the control sample that the soy and hemp treatments considerably decreased cholesterol by 80 and 75, respectively ($P<0.05$). Table 2 demonstrated that, at the probability level, many treatments had a statistically significant effect on HDL value ($P<0.05$). The control treatment showed the most changes. The soybean treatment differed significantly from the other treatments ($P<0.05$). Other treatments do not significantly differ from one another ($P<0.05$).

Furthermore, the other samples did not differ from one another statistically significantly, with the exception of the samples that received palm oil. Triglyceride levels were highest in the control treatment and lowest in the palm oil treatment, respectively.

The key factor contributing to the oxidative stability of oils, according to previous research, is the high level of effective compounds, such as antioxidants, found in oils derived from vegetable seeds. Palm oil is anticipated to exhibit very high thermal stability in the thermal process since it has the highest proportion of saturated fatty acids and the lowest level of polyunsaturated fatty acids. Similar research revealed that palm olein contained 6.03 of polyunsaturated fatty acids and 48.8 of saturated fatty acids (Farhosh *et al*., 2009). The least thermal stability is anticipated for hemp oil, which contains 15.3% linolenic acid. The lowering of linoleic acid, as stated by Warner and Glynn, enhances the oxidative stability of oil (Parsai *et al*., 2017). The quantity of effective compounds and the stability of vegetable oils are positively correlated, according to the available evidence. Moreover, phenolic compounds, which are commonly present in plants, have a significant role in the stability of oils. Majd *et al*. (2021) demonstrated that the use of natural antioxidants in the stability of soybean oil has boosted its oxidative stability. Combining soybean oil and plant essential oils improves the oil's antioxidant activity and oxidative stability (Majd *et al*., 2021). Furthermore, according to Gutfinger (2019), there is a strong correlation between the overall quantity of phenolic compounds in oils (associated with high antioxidant activity) and the Rancimet-measured oxidative stability (Ankica *et al*., 2022).

The milliequivalents of peroxide present in one kilogram of fat are known as the peroxide number. According to Lee's method or the international approach, there should be less than a 5 or a 10 peroxide number in oil and fresh fatty substances. The oil or fatty material is useless if the peroxide number is 20 milliequivalents per 1 kg. Oil extraction methods and storage conditions for oil seeds both have an impact on peroxide number. The amount of peroxide compounds in oil samples increases as it is stored at room temperature (Wu *et al*., 2022). The Codex standard allows for a maximum peroxide content of 10 meq/kg for crude oils. Since hemp contains a larger proportion of saturated fatty acids than soybeans, the peroxide number has changed less in the treatment of the soybean group (Andrade *et al.*, 2022). The hemp seed oil's peroxide number was 0.97 meq/kg (Esmailzadeh *et al.*, 2020). In a similar study, soybean oil had a peroxide value of 1.01 meq/kg, and the low peroxide value was caused by low unsaturation and the processing conditions (Hadadi *et al.*, 2019). The proportion of solid fat and fat crystals in an oil or an oil combination has a large influence on the suitability of that fat for a certain application when analyzing the fatty acid profile. The proportion of solid fat in margarines, shortenings, and fat spreads affects numerous product attributes, including general appearance, ease of packaging, spread ability, oil oxidation, and organoleptic features. Linoleic and oleic acids made up the majority of the fats in soybean oil (Majd *et al.*, 2021). The fatty acid content of oils and the variation in saponification numbers are closely related. Oils having high numbers of long-chain, high molecular weight acids, like linoleic and oleic acids, have lower levels of saponification (Poorfallah *et al.*, 2013). Based on the current investigation, the saponification number grows along with the oxidation resistance time. The findings are in line with those of (Farhoosh *et al.*, 2018). The iodine number is the proportion of iodine absorbed by the double bonds of fatty acids in 100 grams of oil when analyzing quality attributes in oils. The iodine value of the oil sample increases with the degree of unsaturation. According to the standard, frying oils should have an iodine value of no more than 100, and palm oil meets this requirement. Shabani (2019) reported that iodine content was found to be 142.87 in soybean oil with high oleic acid (Li *et al.*, 2022).

One of the dimensionless constants of oils and fats is the refractive index, which is utilized to determine the type of fat as well as any adulteration. The refractive index increases as the chain's length and the quantity of double bonds increase. Omoa *et al.* (2019) reported hemp oil content as 30.5%. The percentage of oil depends on factors such as plant variety, plant cultivation, growth conditions, oil extraction method, and the type of oil solvent used (Omoa *et al.*, 2019). Hemp seed contains the greatest proportion of oil among other oilseeds when compared to soybean oil (20–18%) and other oil sources like cotton seed (20–18%), sunflower (45–34%, and flower (30–35%). By analyzing the physicochemical characteristics of 10 different soybean varieties, Elhami Rad (2018) came to the conclusion that there was a significant difference in the specific gravity of the extracted oils, which ranged from 0.917 to 0.921. The density, molecular weight, and carbon number of the fatty acid chain that makes up the oil are all related to the refractive index, which rises as the molecular weight and saturation level of the oil rise. The extracted oils' refractive indices ranged from 1/4718 to 1/4725 (at 25 degrees Celsius), and they were all within the normal range.

The second section covered the effects of various concentrations of hemp oil, soybean oil, and palm oil on rat blood parameters. The Hemoglobin Dispersion Range (HDW) test reveals how hemoglobin is distributed throughout the blood. According to CV, the typical range for hemoglobin domain distribution is 2.2 and 2.3%. It deals with the uniformity and non-uniformity of hemoglobin distribution in red blood cells. An increase in HDW indicates hemoglobin content heterogeneity in red blood cells, as seen in iron deficiency anemia and sideroblastic anemia. Based on the normal limit of the hemoglobin dispersion factor, all of the samples are found to be within the normal range. It may be inferred that palm oil treatments, which have a larger numerical mean in the distribution diagram, enhance the uniformity of hemoglobin distribution in red blood cells ($P<0.05$). Similar conclusions were achieved by Albrahim *et al.* (2022) when examining the effects of using palm and olive oil, Qiao *et al.* (2022), Gurumallu *et al.* (2022), and Emam *et al.* (2022) while examining the effects of using vegetable oil on blood parameters in mice.

The body needs a certain quantity of cholesterol, which must be naturally present. This substance is crucial for keeping the cell membrane healthy and producing hormones. There are typically no distinct symptoms associated with high cholesterol levels. Excessive blood cholesterol causes plaque to build up in the arteries, which in turn results in side effects including arteriosclerosis, arterial hardening, and heart attacks. The body uses cholesterol for a variety of purposes, including cell growth. Lipoproteins, a type of proteins, carry cholesterol throughout the circulation. As the high-density lipoprotein reaches the liver, the liver converts the low-density lipoprotein into bile, breaks it down, and excretes it from the body. The results of the analysis of variance demonstrated that the treatments significantly affected blood cholesterol levels ($P<0.05$). The control sample makes it clear that the soy and hemp treatments greatly decreased the quantity of cholesterol, with values of 80 and 75 ($P<0.05$). In the study of the impact of soybean oil on cholesterol levels, Zaazaa *et al.* (2023), Yang *et al.* (2022), and Kleckner *et al.* (2022) achieved similar findings. The high-density lipoprotein (HDL) test determines how much healthy cholesterol is present in the blood. In comparison to the control therapy, blood cholesterol significantly decreased when hemp, soybean, and palm oil were used. Similar findings were found in the studies of the impact of palm oil consumption on the level of HDL factor in the blood by Abdulwaliyu *et al.* (2023) and Arias *et al.* (2023).

The tiniest blood cells, known as platelets or plt, help coagulate the blood and stop bleeding when an injury occurs. One drop of blood contains tens of thousands of platelets. Thrombocytopenia is a disorder that occurs when there are less than 150,000 platelets per microliter of blood. In this case, the body has trouble forming a clot, which increases the risk of severe bleeding when an injury occurs. Thrombocytosis happens when there are over 450,000 platelets per microliter, which raises the chance of an unexpected clot or possibly a stroke. The natural range of this factor indicates that the usage of oils has significantly increased the quantity of platelets. When various treatments were examined, it was discovered that only the control treatment was within the normal range, while the other treatments significantly increased the amount of blood platelets, which is thought to be harmful to health.

The body instantly stores wasted calories in the form of triglycerides, a kind of lipid or fat. Moreover, it supplies the blood with the energy required for muscular activity. Following a meal, too many triglycerides enter the bloodstream. The triglyceride level rises if more calories are ingested than the body needs. Except for the samples with a diet in palm oil, none of the other samples showed any statistically significant changes. The outcomes of all treatments were within the normal range for fasting triglyceride levels, which should generally not exceed 150 mg per deciliter. Fleke *et al.* (2022) and Shcherbakova *et al.* (2023) obtained similar results in the study of the effect of oil and fat on triglyceride levels.

Conclusions and Recommendations

The human diet cannot exist without edible oils, which are often ingested either directly and pure or as a component of food. Hence, it is crucial to study the physicochemical characteristics of oil and its period of stability in comparison to oxidation and fatty acid components. The findings showed that compared to palm and hemp oils, the physicochemical changes in soybean oil was more beneficial. All oils had the same refractive index, and palm oil had stronger oxidative stability than hemp and soybean oil. Linoleic acid were found to be the most prevalent fatty acids when the fatty acid profile was examined for soybean and hemp oils, respectively, whereas it was oleic acid for palm oil. It may be noted that the usage of vegetable oils with high amounts of unsaturated fatty acids in the diet of rats played a role in lowering cholesterol and HDL when looking at the effect of consuming palm, hemp, and soy oils in the diet of rats. Compared to the control sample, it results in a rise in blood platelet levels. This concentration in the soybean oil sample is over the allowable limit.

Novelty Statement

The analysis examines physicochemical characteristics, oxidative stability, and rat blood parameters of soybean, palm, and hemp oils, revealing potential health benefits and differences. Further investigation is needed for dietary recommendations and applications

Author's Contribution

Maryam Beheshti: Done this research and designed the study and wrote the first version of the report.
Mohammad Reza Taheriyan: Analyzed the data and Supervision of the Student.
Sikander Shahzad and Memoona Siddique: They review the introduction section.
Muhammad Farooq: Conceptualization, Review the manuscript and amp; editing.
Huzaifa Kashif and Naila Ilyas: Help in review the article.

Conflict of interest
The authors have declared no conflict of interest.

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