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# INVESTIGATING THE ANTIOXIDANT POTENTIAL OF PEA POD EXTRACT FOR IMPROVING OXIDATIVE STABILITY OF SUNFLOWER OIL

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## ABSTRACT

Oxidation in edible oil and fat is one of the main problems that fat and oil industry is facing. Due to human health concerns, synthetic antioxidants usage in food products is severely controlled. The present study was conducted to evaluate the capability of pea pod extract to prevent oxidation in sunflower oil. Pea pods were collected and sunflower oil was procured from local market. After extraction, extract was subjected to antioxidant activity analysis (FRAP assay and DPPH assay) and physicochemical analysis. Fatty acid profile of extract was determined by GC-FID. Then pea pod extract was incorporated in commercially available sunflower oil at the concentration of 600ppm, 800pp, 1000ppm and 1200ppm. During 60 days of storage all blended oil samples, were subjected to physicochemical analysis to assess the oxidative stability. During 60 days of storage FFA value increased and it ranged from 0.18±0.03 to 0.46±0.05, saponification value increase it ranged from 193.36±1.60 to 200.39±1.78. Peroxide value increased and it was within range of 1.96±0.07 to 11.44±0.95. The overall results indicate that the pea pod extract has high antioxidant activity and it has improved the oxidative stability of sunflower oil during 60 days of storage. Sensory evaluation showed that among all treatments $T_3$ got the highest sensory attributes and was selected as best one.

## INTRODUCTION

Fats and oils are the primary source of energy, fat-soluble vitamins and essential fatty acids. (Kumar *et al.,* 2020). In storage of processed edible oils and packaged edible oils oxidation is key factor that affects stability (Kaleem *et al.,* 2015). Sunflower is one of the popular oil seed that consist of consequential oil content with huge quantity (50-60%) of lipid and protein (Hanan *et al.,* 2020). In contrast with other vegetable oil sunflower oil also comprises an appreciable amount of vitamin B complex, vitamin D and K as well as ample quantity of lipid and protein content (Franco *et al.,* 2018). After extraction of sunflower oil remaining part of oil seed is called sunflower meal which is a promise source of protein (Islam *et al.,* 2018). Sunflower is one of the best edible a rich in oleic 14.10-18.1% and linoleic 59-67.5% unsaturated fatty acid (Fozia *et al.,* 2018).

The legume pea, which belongs to the *Leguminoceae* family, is the most eaten legume worldwide. Green peas are abundant source of vitamins, minerals and fibers and have greater value for health (Dahl *et al.,* 2012). The usage of the different techniques like peeling, washing, freezing result in the reduction of the pesticide residue (Kaushik *et al.,* 2009). Pea seeds have been found to have anti-diabetic, antibacterial and antioxidant properties due to their rich nutritional content, including carbohydrate, crude protein, lipid, ash, dietary hull fibers, monosaturated fatty acids, polyunsaturated fatty acids and vitamins i.e. K, Mg, Ca, Na, Fe, Zn (Mejri *et al.,* 2019). Pea fiber improves gastrointestinal health by reducing starch digestibility, while transitory amylose capacity aids in a lower glycemic index (Haymanti *et al.,* 2014).

Oxidation makes product unfit by disintegrating the nutritional quality, followed by generation of toxic substances (Azman *et al.,* 2020). Different techniques are used to prevent oxidation such as inhibition of oxygen access, inactivation of enzyme catalyzing oxidation, utilization of suitable packaging and decrease of oxygen pressure (Urbancic *et al.,* 2019). Partial hydrogenation of fats and oils can be used to prevent rancidity of edible oil because it eliminates the chances of oxidation by removing high unsaturated fatty acids that are highly susceptible to oxidation(Jalili Safaryan *et al.,* 2016). Researches have revealed that trans fats are bad fats and very atherogenic as compare to saturated fats because they lower the high-density lipoprotein (HDL) which is decent for health and rise low density lipoprotein (LDL) that is harm for health. Most common methods used to prevent rancidity are addition of antioxidants and control peroxidative metals (Decker *et al.,* 2016).

Lecithin is separated from crude oil during the process of degumming; it is used as food antioxidant in different food products. Deodorization is the step of the refining process of edible oil (Kamboj and Nanda, 2018). Tocopherols are appropriate antioxidants and phytosterols have ability to lower cholesterol in blood serum(Castaldo *et al.,* 2021). Tocopherol obtained from deodorization concentrates are more useful compare to synthetic α-tocopherol because they are natural blend of antioxidants, that are optically active and demonstrates their natural origin (Pokorny and Korczak, 2015). (Upadhyay and Mishra, 2015).

Synthetic antioxidants which are mostly utilized in commercially available edible oils are butylated hydroxy toluene (BHT), tertiary butyl hydroquinone (TBHQ) and butylated hydroxyl anisole (BHA) (Islam *et al.,* 2018). Several studies have done on the application of synthetic antioxidants that showed these antioxidants can be a reason of hazard to the health of human such as DNA injury and carcinogenesis (Sikwese and Duodu, 2017). BHT and BHA also responsible for liver damage (Sayyad and Farahmandfar, 2017).

## METHODOLOGY

### Preparation of pea pod powder

Dry pea pods were rinsed into the water to remove impurities and dirt particles. Pea pods were soaked for 8-12 hours. After the resting time the pea pods were dehydrated in the oven. The dry material was ground to fine powder with the help of a laboratory grinder and was pass through 80 meshes size sieves.

### Proximate analysis of pea pod powder

Proximate analysis of pea pod powder includes moisture, ash, fat, protein, fiber and nitrogen free extract were performed.

### Moisture Content

Moisture content of pea pod powder was determined using the methodology suggested in AOAC (2023). In a china dish 5g of sample was added. China dish along with the pea pod powder sample in it were weighed and the reading was noted. Then china dish was placed in a hot air oven at 105 °C for 24 hours. After that sample was taken out of the oven placed in the desiccator in order not to gain moisture again from the atmosphere and removed from the desiccator until cooled down and weighed the china dish again. Moisture content in the sample was calculated by the given formula:

$$\text{Moisture(\%)} = \frac{\text{Wt. of sample(g) - Wt. of sample after drying (g)}}{\text{Wt. of sample (g)}} \times 100$$

### Ash Content

Measurement of ash content of pea pod powder was done by using the methodology suggested in AOAC (2023). First of all 10g of the sample was taken in the dry clean crucible and weight the sample. The content of ash was investigated at high temperature after incineration in the muffle furnace. When the sample was incinerated the moisture comes out and the organic material such as protein, oil and starch burn and ash was left behind. The sample was placed in the muffle furnace at the temperature of about 585°C for 4-6 hours. Then the remaining material was cooled by placing it at room temperature. The ash content indicates the overall content of minerals present in the sample. The content of ash in pea pod powder was calculated by the following formula:

$$\text{Ash(\%)} = \frac{\text{Wt. of ash sample (g)}}{\text{Wt. of sample (g)}} \times 100$$

### Crude Protein

Protein content of pea pod powder was determined according to the protocols as described in AOAC (2023) by using Kjeldhal equipment. Pea pod powder 2g was weighed and added into the digestion flask along with the addition of 25.0 mL sulphuric acid and digestion tablet. Then digestion flask was placed in a fume hood for digestion. Digestion flask was removed from the fume hood when the color changed. Then cooled down the sample and 150ml of distilled water was added. Distillation was done by taking the 10mL of digested sample in a conical flask and added 40% 10 mL NaOH. Ammonia ($NH_3$) gas was released during this procedure and added 2-3 drops of methyl red indicator, 4% boric acid solution trapped in it. Continued the process for about 3 to 4 minutes until the change in color of boric acid was noticed and the maximum amount of ammonia was collected. Titration of the distillated sample was done by using 0.1N $H_2SO_4$ in the flask until the golden yellow color appeared and noted the reading. The protein content of pea pod powder was calculated by the following formula:

$$\text{Protein(\%)} = \frac{\text{Volume of } H_2SO_4 \text{ used} \times 0.0014 \times \text{Dilution volume (250ml)}}{\text{Wt. of initial sample (g)} \times \text{Volume of diluted sample used (ml)}} \times 100$$

Protein (%) = % N x 6.25

### Crude Fat

Fat content in pea pod powder was determined by using the methodology suggested in AOAC (2023). First of all 10g sample of pea pod powder was dried in the hot air oven. Then properly weigh the solvent extraction thimble (filter paper). Then place the sample into filter paper and again weight the thimble with the sample. After that dry the soxhlet extraction flask and extraction of crude fat was performed by using hexane condensing 5 to 6 drops per second in soxhlet extraction unit. When no odor was retain then dry the flask at 100°C for 30 minutes in the hot air oven. The flask was cooled in the desiccator and weight properly.

**Crude Fiber**

It was determined according to the protocols as described by AOAC (2023). Weighed 2-3g of sample into a beaker that had a capacity of 1L, 200ml of 1.25% sulphuric acid was added into the beaker and boiled it for about thirty minutes. To obtain an acid-free sample, hot water was used to rinse the residues after boiling. These residues were then transferred into another beaker with the capacity of 500ml and 1.25% NaOH was added and boiled for about 30 minutes. After boiling the residues were washed with hot water in order to get alkali free sample. After this step, the crucible was weighed and reading was noted and then transferred these residues in crucible. Put it into oven at 100°C for almost 3 to 4 hours for drying sample. After completion of time weight was noted by using weighing balance. Then crucible was placed into muffle furnace at the temperature of 500 °C until greyish colored material was obtained and after it cooled down, weighed it and note the reading.

**Nitrogen Free Extract**

The Nitrogen free extract of pea pod powder was calculated by the following formula:

NFE (%) = 100-[Moisture (%) + Ash (%) + Protein (%) + Fat (%) + Fiber (%)]

**Extraction of pea pod extract**

Pea pods were cleaned and partially dried for seven days followed by oven drying at 105 °C for a period of 4 hours or until constant weight. Oil was extracted through ultrasound assisted extraction technique by using ethanol as a solvent. After extraction, it was filtered through filter paper to remove coarse particles.

**Physicochemical Analysis of pea pod extract**

**Free fatty acids**

The measurement of free fatty acids present in pea pod extract was estimated according to the standard method suggested in AOCS (2018). The sample of about 10ml of pea pod extract was taken in a conical flask then 25ml ethanol was pour into it and mixes it well until the oil sample was miscible in the ethanol. Then 2-3 drops of phenolphthalein indicator was added and shake vigorously and 0.1N NaOH was used to titrate the mixture with constant stirring until the pink color was achieved. The free fatty acid (FFA) value of pea pod extract was calculated by the following formula:

$$\text{Free Fatty Acid}(\%)=\frac{\text{Alkali used(ml)}\times \text{N of alkali}\times 28.2}{\text{Weight of sample (g)}}$$

**Peroxide value**

The peroxide value of pea pod extract was calculated according to the standard method suggested in AOCS (2018). First of all 5ml of pea pod extract sample was taken into a 250ml of conical flask. Then 30ml of acetic acid-chloroform (3:2) solvent mixture was added and whirled for approximately 1 minute until it was dissolved. After that, about 0 to 5ml of standard potassium iodide solution was added with pipette. This mixture was stand in cool and dark place. After that, titrate the sample solution against the 0.1N $Na_2SO_3$; sodium thiosulphate solution. Continuously stir the solution until the yellow color of the mixture was appeared. After vigorous shaking, 0.5ml of starch solution was added as indicator for changing the color of the solution to blue. Keep the shaking continuous until the blue color disappeared. The peroxide value (PV) was calculated by the following formula:

$$\text{Peroxide value (meq/kg)}=\frac{\text{(B-S)}\times \text{N}\times 1000}{\text{Weight of oil (g)}}$$

**Specific Gravity**

The specific gravity of pea pod extract was estimated according to the procedure of AOCS (2018). First of all, fully dry the pycnometer. Then it was filled with the oil sample in such a manner that air bubble entrapment was avoided when the side arm cap was removed. The stopper was inserted and pycnometer was placed into the water bath for 30 minutes. The cap was taken off from the side arm and immediately weight the sample accurately. Specific gravity of pea pod extract was computed by the following formula:

$$\text{Specific gravity}(\text{g/cm}^3)=\frac{\text{Weight of the oil sample (g)}}{\text{Weight of water (g)}}=\frac{\text{C - A}}{\text{B - A}}$$

**Iodine value**

The Iodine value of pea pod extract was estimated according to the procedure of AOCS (2018). The pea pod extract sample of about 5ml was taken in the Erlenmeyer flask after that, add about 25ml of carbon tetrachloride (CCL) solution and continuously stir the sample followed by the addition of Wij's solution. All the content should be well mixed. Then the solution was allowed to stand for approximately 30 minutes in a dark place. The distillation was performed by using the 10% of potassium iodide solution and 100 ml of boiled and cooled distilled water. Then the flask contents were titrating against 0.1N of sodium thiosulphate and starch solution was used as an indicator in this phenomenon. A blank reading was also conducted in such a manner and the given formula was used to calculate the iodine value of pea pod extract.

$$\text{Iodine value}(gI_2/100g) = \frac{(B - S) \times N \times 12.69}{\text{Weight of the sample (g)}}$$

**Refractive index**

The refractive index of pea pod extract was determined by following official method described by AOCS (2018). Set refractometer temperature to 40°C for oil samples. Following that, a few drops of sample were deposited on the lower prism. Then prism was closed and tightened securely with screwhead. The sample was then allowed to stand for roughly 1-2 minutes, or until it reached the temperature of the sensor. After adjusting the instrument and light to produce the most distinct reading possible, the refractometer reading was recorded.

**Fatty acid profile by GC-FID**

Gas chromatography technique was used for classification of triglycerides described by Carvalho *et al.* (2012). The analysis was done on a gas chromatograph fitted with flame ionization detector (FID). Flame ionization detector would work by flame to ionize the carbon present in organic compounds. Separation was held in the column (DB WEX 30M 0.25) and analyte passed through a flame that had been fueled by nitrogen and air resulted in ionization of carbon. The temperature of the injector was kept at 250°C and sample quantity was 2 ml, the velocity was kept at 30 cm/s and $N_2$ was used as carrier gas with the 30 ml/min flow rate. The temperature of the oven started at 140°C and it was further brought to 240°C with the rate of 4°C per minute. The temperature was kept at this for 5 minutes. The temperature of the detector was kept at 260°C. The pea pod extract fatty acid profile was determined by the ratio between the area and mass of inner standard and the area of the recognized fatty acid. The concentration of the fatty acids was depicted by mg.

**Antioxidant potential test**

**DPPH Radical Scavenging Assay**

DPPH radical scavenging assay was done according to the procedure of Olszowy and Dawidowicz (2016). The 3ml of the samples were taken which were diluted with the help of methanol. Then these samples were subjected to be mixed with the DPPH (3ml) in methanol ($2.0 \times 10^{-4}$) that was the final concentration of DPPH obtained. After that, the process of incubation was performed for approximately 30 minutes for all the samples and these were vigorously shaken before incubation. Absorbance of the prepared solution was run at 515nm alongside a blank solution using spectrophotometer.

**Ferric reducing antioxidant power (FRAP) assay**

The ferric reducing antioxidant power was performed by following method of Olszowy and Dawidowicz (2016). The FRAP reagent was prepared using 25ml of 0.1mol/L acetate buffer with pH 3.6, 2.5ml mmol/L FeCk, and 2.5ml of 10mmol/L TPTZ solution in 40mmol/L of HCI and incubated at 40°C for around 50 minutes. Then, in a volumetric flask, 0.1-0.3ml of the sample extract or standard were mixed with 2ml of FRAP reagent and filled with distilled water to the required level. The obtained blue solution was kept at room temperature for about 10 minutes before being centrifuged at 5000rpm for 20 minutes. The absorbance at 593nm was then evaluated using a spectrophotometer.

**Preparation of Sample**

Commercially available sunflower oil was blended with pea pod extract at different concentrations ranging from 600ppm to 1200ppm per 100 ml oil. Synthetic antioxidant BHT was added at 200ppm to compare the competence of the natural antioxidants.

**Table 1: Treatment plan for pea pod extract in sunflower oil**

| Treatment | Pea pod extract (ppm) | BHT (ppm) |
|---|---|---|
| $T_0$ | - | - |
| $T_1$ | - | 200 |
| $T_2$ | 600 | - |
| $T_3$ | 800 | - |
| $T_4$ | 1000 | - |
| $T_5$ | 1200 | - |

$T_0$ = Control, Sunflower oil without any antioxidant

$T_1$= 200 ppm concentration of synthetic antioxidant BHT

$T_2$= 600 ppm concentration of pea pod extract

$T_3$= 800 ppm concentration of pea pod extract

$T_4$= 1000 ppm concentration of pea pod extract

$T_5$= 1200 ppm concentration of pea pod extract

**Physicochemical analysis of extract added sunflower oil**

**Saponification value**

Saponification value of sunflower oil was determined using the methodology suggested in AOCS (2018). It was confirmed that oil sample was fully dry then weight 2.0g of oil sample. This oil sample was put into the Erlenmeyer flask of 250ml. Then oil sample was completely mixed with the alcoholic potassium hydroxide solution (KOH). The alcoholic potassium hydroxide (KOH) content that was used to determine the saponification value was about 25ml and it was added slowly into the sunflower oil sample. Then this sample was reflux in water bath for about 30 minutes. Sample was shaking vigorously followed by addition of about 2-3 drops of phenolphthalein indicator. Then it was treated with the 0.5N of hydrochloric acid (HCL) until the pink color changes to the clear solution, this clarity was achieved within one hour of boiling. Blank determination was also done along with sample determination. The saponification value of sunflower oil was calculated by the given formula:

$$\text{Saponification value (mg/g)} = \frac{56.1\,(B - S) \times N \text{ of HCL}}{\text{Weight of the sample (g)}}$$

**Free fatty acids**

The measurement of free fatty acids present in sunflower oil was estimated according the standard method suggested in AOCS (2018). The sample of about 10ml sunflower oil was taken in a conical flask then 95% of 25ml ethanol was pour into it and mix it well until the oil sample was miscible in the ethanol. Then 2-3 drops of phenolphthalein indicator was added and shake vigorously and 0.1N NaOH was used to titrate the mixture with constant stirring until the pink color was achieved. The free fatty acid (FFA) value of sunflower oil was calculated by the following formula:

$$\text{Free Fatty Acid}(\%) = \frac{\text{Alkali used(ml)} \times \text{N of alkali} \times 28.2}{\text{Weight of sample (g)}}$$

**Peroxide value**

Peroxide value of sunflower oil was calculated according to the standard method suggested in AOCS (2018). First of all 5ml of sunflower oil sample was taken into a 250ml of conical flask. Then 30ml of acetic acid-chloroform (3:2) solvent mixture was added and whirled for approximately 1 minute until it was dissolved. After that, about 0 to 5ml of standard potassium iodide solution was added with pipette. This mixture was stand in cool and dark place. After that, titrate the sample solution against the 0.1N $Na_2SO_3$; sodium thiosulphate solution. Continuously stir the solution until the yellow color of the mixture was appeared. Subsequently, the starch solution was used as an indicator for changing the color of the solution to blue. Keep the shaking continuous until the blue color disappeared.

**Specific Gravity**

First of all, fully dry the pycnometer. Then it was filled with the oil sample in such a manner that air bubble entrapment was avoided when the side arm cap was removed. The stopper was inserted and pycnometer was placed into the water bath for 30 minutes.

The pycnometer was completely cleaned and dry and cap was removed from the side arm and removes it from the water bath. The cap was taken off from the side arm and immediately weight the sample accurately. Specific gravity of sunflower oil was computed by the following formula:

$$\text{Specific gravity}(g/cm^3) = \frac{\text{Weight of the oil sample (g)}}{\text{Weight of water (g)}} = \frac{C - A}{B - A}$$

**Iodine value**

The Iodine value of sunflower oil was estimated according to the procedure of AOCS (2018). The sunflower oil sample of about 5ml was taken in the Erlenmeyer flask after that add about 25ml of carbon tetrachloride (CCL) solution and continuously stir the sample followed by the addition of Wij's solution. All the content should be well mixed. Then the solution was allow to stand for approximately 30 minutes in a dark place. The distillation was performed by using the 10% of potassium iodide solution and 100 ml of boiled and cooled distilled water. Then the flask contents were titrate against 0.1N of sodium thiosulphate and starch solution was used as an indicator in this phenomenon.

**Smoke point**

Fill the cup with oil such that the top of the meniscus is completely aligned with the filing line and adjust the apparatus position so that the light beam crosses the middle of the cup. Suspended or mounted the thermometer vertically in the dish so that the bottom of the bulbs was about 6mm above the bottom of the cup. The material was then rapidly heated to roughly 42°C of smoke point. After that, the heat was regulated such that sample temperature rises at rate of 5-6°C/min. The temperature at which the sample continuous stream of bluish smoke which is referred to as smoke point was measured by the thermometer immersed in the oil sample.

**Refractive index**

Set refractometer temperature to 40°C for oil samples. Following that, a few drops of sample were deposited on the lower prism. Then prism was closed and tightened securely with screwhead. The sample was then allowed to stand for roughly 1-2 minutes, or until it reached the temperature of the sensor. After adjusting the instrument and light to produce the most distinct reading possible, the refractometer reading was recorded. The average of all the values was obtained after many readings were taken.

**Antioxidant potential test**

**DPPH Radical Scavenging Assay**

DPPH radical scavenging assay was done according to the procedure of Olszowy and Dawidowicz (2016). The 3ml of the samples were taken which were diluted with the help of methanol. Then these samples were subjected to be mixed with the DPPH (3ml) in methanol ($2.0 \times 10^{-4}$) that was the final concentration of DPPH obtained. After that, the process of incubation was performed for approximately 30 minutes for all the samples and these are vigorously shaken before incubation. Absorbance of the prepared solution was run at 515nm alongside a blank solution using spectrophotometer. The DPPH scavenging activity was measured using formula:

$$\text{Reduction of absorbance (\%)} = \frac{AB - AA}{AA} \times 100$$

AB= Absorbance of blank

AA= Absorbance of sample extract

**Ferric reducing antioxidant power (FRAP) assay**

The FRAP reagent was prepared using 25ml of 0.1mol/L acetate buffer with pH 3.6, 2.5ml mmoL/L FeCk, and 2.5ml of 10mmol/L TPTZ solution in 40mmol/L of HCI and incubated at 40°C for around 50 minutes. Then, in a volumetric flask, 0.1-0.3ml of the sample extract or standard were mixed with 2ml of FRAP reagent and filled with distilled water to the required level. The obtained blue solution was kept at room temperature for about 10 minutes before being centrifuged at 5000rpm for 20 minutes. The absorbance at 593nm was then evaluated using a spectrophotometer.

**Storage stability**

After adding pea pod extract the storage stability of sunflower oil was analyzed at 0, 15, 30, 45 and 60 days.

**Frying Stability**

Frying stability of the treated sunflower oil was checked with the best selected treatment level by developing a product i.e. french fries.

**Sensory analysis**

The 9-point Hedonic Score System was used to assess the consumer acceptance of French fries for color, flavor, taste, texture and overall acceptability, as reported by Meilgaard *et al.* (2007). Faculty members and postgraduate students from NIFSAT participated in the evaluation to choose the best treatment for French fries.

**Statistical analysis**

Data was subjected to appropriate tool to find out the level of significance as described in Montgomery (2017). For the purpose of determine the comparison between the mean values and to find out the level of significance statistical analysis were done on the data that was obtained from each parameter.

## RESULTS

### Proximate analysis of pea pod powder

**Moisture**

The moisture content of the pea pod powder was determined by AOAC (2023). Higher chances of microbiological attack. Moisture (%) of pea pod powder demonstrates the moisture (%) of pea pod powder 10.78±0.39%. Rudra *et al*. (2020) investigated that the moisture (%) of pea pod powder was 10.02% and Nasir *et al*. (2023) reported that the moisture (%) of pea pod was 6.3%.

**Ash test**

The ash (%) of pea pod powder was determined by AOAC (2023). The organic compounds were burn and the inorganic contents remain left which shows the presence of minerals in the pea pod powder. Ash (%) of pea pod powder in the pods was 4.95±0.25%. Rudra *et al*. (2020) reported that the ash (%) of pea pod was 4.61 % and Nasir *et al*. (2023) demonstrated that the ash (%) of pea pod was 5.2%.

**Crude Protein**

The determination of protein content of pea pod powder was done by AOAC (2023) and it depends upon the oxidation of organic components with the help of sulphuric acid in the presence of catalyst. The value of pea pod protein was 16.67±0.73% Rudra *et al*. (2020) demonstrated that the content of protein of pea pod was 11.99 % and Nasir *et al*. (2023) reported that the protein content of pea pod was 13.3%.

**Crude fat**

The determination of crude fat content was done by AOAC (2023). The crude fat indicates the presence of crude mixture of fat soluble material in the sample. The crude fat was extracted via soxhlet from about 10g of dried pea pods powder. Extract was evaporated from the dried sample and the residue was weighted and reported as fat. The value of crude fat of pea pod was 0.43 ±0.01%.

**Crude fiber**

The estimation of crude fiber content was done by AOAC (2023). It may also be known as the quantity of indigestible lignin, cellulose several other components are present in the food. These constituents have a little bit of food value but these may provide the bulk essential for the proper peristaltic action. 10 grams of pea pod powder sample was taken and dietary fiber content was estimated via fiber tech and the crude fiber content of the selected sample was 26.56±0.18%.

### Physicochemical analysis of pea pod extract

**Free fatty acid value**

Free fatty acid determined the purity as well as the freshness of the oil. If the fatty acid content is high in the fats and oil then it is affirm that this oil will be more susceptible to oxidation. This proliferation response and hydrolysis of triglycerides of fats and oils in the presence of water is a cause of formation of free fatty acids.

The free fatty acid value of pea pod extract was estimated by the method that was suggested in AOCS (2018). The free fatty acid value of pea pod extract was 6.23±0.11%, this value was also compared with the value of soybean oil 0.21±0.02% that was estimated by Olagunju *et al.* (2022).

**Peroxide value**

Peroxide value is an analysis for oil oxidative rancidity, because it measures the amount of primary oxidation products like peroxide and hydroperoxides produced during the early stages of oil and fat oxidation. Peroxide value is the amount of reactive oxygen in milliequivalents of free iodine per kg of fat. The peroxide value of pea pod extract was 2.37±0.19 meq/kg that was determined by using the method describe in AOCS (2018).

**Specific gravity**

The specific gravity is basically used to estimate the purity of the fats and oils. Usually, it is considered that the specific gravity is marker for the clarity of edible fats and oils. The value of specific gravity always lies below and the normal value lies between 0.885-0.959 g/cm³ for fats and oils.

The value of specific gravity was 0.885±0.005 g/cm³ that determined by the method which was described by AOCS (2018) by using the pycnometer and by maintaining the temperature of 25°C of pycnometer and the water bath.

**Iodine value**

There are numerous analytical procedures that are used to calculate the iodine value and the purpose of estimation of iodine value is to estimate the unsaturation in the fatty acids constituents. The iodine value can also be defined as the iodine number which is used up by hundred gram of fats and oil. The iodine value of pea pod extract was 123.51±6.17 g/100g of oil and which was determined by the method describe in AOCS (2018).

**Refractive Index**

The ratio of speed of light in a vacuum to the speed of light in a sample medium is how refractive index is defined. A medium's refractive index is determined by its chemical composition. As the light travels via the interface of two mediums with various indices, it bends. As a result, the refractive index values are always greater than 1. The refractive index of the oils is influenced by the degree of unsaturation, molecular weight, degree of degradation and fatty acid chain length. The refractive index, which increases as oil thickness increases, is another tool to measure oil thickness.

In this study the refractive index 1.458±0.007 of pea pod extract was discovered.

**Table 1: Physicochemical analysis of pea pod extract**

| Physicochemical analysis | Mean value |
|---|---|
| FFA (%) | 6.23±0.11 |
| Peroxide value meq/kg | 2.37±0.19 |
| Specific gravity g/cm³ | 0.885±0.005 |
| Iodine value (g $I_2$ /100 g) | 123.51±6.17 |
| Refractive Index | 1.458±0.007 |

**Antioxidant activity analysis**

**DPPH assay**

DPPH assay determined the scavenging activity of DPPH radical which inhibit oxidation of lipids present in a product and provide information related to the scavenging capacity of DPPH radical used in the process. 2, 2-diphenyl-1-picrylhydrazyl (DPPH radical) produces violet color in ethanol solution. The results showed similarity with the findings of Elbadray and Sello (2016) who determined the nutrition profile of tomato peel extract along with antioxidant capacity of the tomato peel.

**FRAP assay**

This method is based on the reducing ability of antioxidant present in sample. The reaction forms a blue chromophore due to reduction of ferric-tripyridyltriazine to ferrous-tripyridyltriazine. This method determines the antioxidant capacity of the natural antioxidants that can be used in food products to enhance the antioxidant activity.

**Table 2: Antioxidant analysis of pea pod extract**

| Test | Quantity |
|---|---|
| DPPH (% inhibition) | 44±0.76% |
| FRAP (μmol TE/mL) | 55±0.84 |

**Fatty acids profile by GC-FID**

Fatty acid is considered as a carboxylic acid that has long aliphatic chain that may be unsaturated or saturated. Most commonly, the unsaturated fatty acids have unbranched chain of the even number (4 to 28) of the carbon atoms.

The results of allocated sample of pea pod extract was compared by the study Mejri *et al*.

(2019) Margaric acid(C17:0) 0.76, Behenic acid(22:0) 1.6, Myristic acid (C14:0) 0.68, Palmitic acid (C16:0) 21, Linoleic acid (C18:2) 35, Stearic acid (C18:0) 5, Oleic acid (C18:1) 3.6, Linolenic acid (C18:3) 29, Arachidic acid (C20:0) 2.49 , Others 0.70, Saturated fatty acids 32, Unsaturated fatty acids 68.

**Table 3: GC-FID Analysis of Pea pod extract**

| Fatty acid profile (%) | Peak Area (%) |
|---|---|
| Margaric acid (C17:0) | 0.74 |
| Myristic acid (C14:0) | 0.66 |
| Palmitic acid (C16:0) | 20.50 |
| Linoleic acid (C18:2) | 34 |
| Stearic acid (C18:0) | 6.30 |
| Oleic acid (C18:1) | 4.15 |
| Linolenic acid (C18:3) | 28.40 |
| Arachidic acid (C20:0) | 2.35 |
| Behenic acid (C22:0) | 1.5 |
| Others | 0.70 |

**Analysis of extract added sunflower oil**

**Peroxide value**

Peroxide value basically determines the oxidation of oil as it causes free radicals that interact with other molecules and produce aldehydes and ketones and due to production of these bodies off product gives off flavor.

By measuring PV in the absence and presence of synthetic antioxidants, as well as pea pod extract as a source of natural antioxidants, at 25°C for 60 days, the degree of oxidation on samples of sunflower oil was evaluated. This study showed that at the end of 60 days of storage time, peroxide value was maximum detected for the control treatment $T_0$ in which 0% pea pod extract and 0% synthetic antioxidant was used. The lowest peroxide value was noticed for the $T_5$ in which 1200ppm pea pod extract was used. These results showed that peroxide value can be reduced by increasing the pea pod extract value because the extract has antioxidative properties. These findings are similar to Khalid *et al.* (2021) who studied the phenolic content and antioxidant activity of apple peel extract and concluded that apple peel extract can be used as excellent and natural source of antioxidant and can be used for increasing shelf life of different food products.

Our results are according to the findings of Iqbal and Bhanger (2007). The peroxide value calculated for samples were between 10.72-18.37 meq/kg for the sample stabilized by natural antioxidant and stored for period of 24 days. While highest peroxide value calculated for control sample was 17.21 meq/kg. During all stages of storage period highest peroxide value was calculated for control sample followed by SFO-250, SFO-500, SFO-BHA, SFO-1000 and SFO-BHT respectively.

**Table 4: Treatment mean for peroxide value (meq/kg oil) of sunflower oil samples treated with pea pod extract**

| Treatment | Storage (Days) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | |
| $T_0$ | 2.27±0.12 | 4.32±0.18 | 7.43±0.24 | 10.76±0.29 | 13.32±0.33 | 7.62$^a$ |
| $T_1$ | 1.93±0.04 | 4.08±0.13 | 6.45±0.19 | 9.32±0.19 | 11.76±0.24 | 6.71$^b$ |
| $T_2$ | 1.98±0.08 | 4.19±0.09 | 7.05±0.28 | 9.89±0.24 | 12.20±0.37 | 7.06$^b$ |
| $T_3$ | 1.95±0.09 | 4.12±0.14 | 6.71±0.21 | 9.65±0.20 | 11.98±0.29 | 6.88$^b$ |
| $T_4$ | 1.88±0.05 | 3.54±0.13 | 5.63±0.17 | 7.80±0.26 | 10.13±0.24 | 5.79$^c$ |
| $T_5$ | 1.74±0.03 | 3.12±0.07 | 5.09±0.13 | 6.94±0.21 | 9.25±0.32 | 5.22$^d$ |
| **Mean** | 1.96$^e$ | 3.89$^d$ | 6.39$^c$ | 9.06$^b$ | 11.44$^a$ | |

$T_0$ = Control, Sunflower oil without any antioxidant $T_1$= 200 ppm concentration of synthetic antioxidant BHT

$T_2$= 600 ppm concentration of pea pod extract $T_3$= 800 ppm concentration of pea pod extract

$T_4$= 1000 ppm concentration of pea pod extract $T_5$= 1200 ppm concentration of pea pod extract

**Free fatty acids**

Triacylglycerol yields fatty acids when ester linkages are broken by moisture, lipase action, and high temperatures. $T_0$ had the highest free fatty acid concentration on 60th day with increasing storage days, the FFA percentage increased in all the samples. At 60th day, all treatments reached their maximum level. Despite that the FFA concentration increased with storage, free fatty acid growth was minimal in pea pod extract treatments. As the percentage of pea pod extract increased the amount of free fatty acid decreased because the pea pod extract has antioxidative properties. $T_5$ and $T_4$ had the lowest mean value, which were lower than the control treatment $T_0$. Pea pod extract was found to be efficient in lowering the fatty acids formation during sunflower oil storage.

**Table 5: Treatment mean for FFA value (%) of sunflower oil samples treated with pea pod extract**

| Treatment | Storage (Days) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | |
| $T_0$ | 0.30±0.03 | 0.44±0.02 | 0.48±0.07 | 0.54±0.09 | 0.62±0.09 | 0.47$^a$ |
| $T_1$ | 0.14±0.02 | 0.16±0.01 | 0.26±0.03 | 0.30±0.07 | 0.40±0.08 | 0.26$^c$ |
| $T_2$ | 0.22±0.07 | 0.24±0.03 | 0.31±0.09 | 0.32±0.06 | 0.48±0.04 | 0.31$^b$ |
| $T_3$ | 0.19±0.02 | 0.21±0.05 | 0.28±0.03 | 0.29±0.02 | 0.46±0.07 | 0.29$^{bc}$ |
| $T_4$ | 0.16±0.03 | 0.18±0.04 | 0.27±0.04 | 0.28±0.05 | 0.45±0.03 | 0.25$^{de}$ |
| $T_5$ | 0.10±0.02 | 0.14±0.02 | 0.25±0.05 | 0.26±0.03 | 0.38±0.05 | 0.23$^e$ |
| Mean | 0.18$^d$ | 0.23$^c$ | 0.31$^b$ | 0.33$^b$ | 0.46$^a$ | |

**Iodine value**

An estimate of melting point and oxidative stability can be derived from the iodine value due to the relationship between degree of unsaturation and these two properties. The findings are often reported in terms of the quantity of iodine that was absorbed for every 100 grams of oil or fat. The results are usually expressed as the amount of iodine absorbed per 100g of the oil or fat. During the 60 days of storage study iodine was analyzed in sunflower oil samples at regular intervals. Lowest iodine value on the 60th day was recorded for $T_0$ (100.32 $I_2$g/100g) as it contain no antioxidant and $T_1$ having synthetic antioxidant had iodine value (109.66 $I_2$g/100g) and the iodine values observed in $T_2$, $T_3$, $T_4$ and $T_5$ were 101.88 $I_2$g/100g, 107.03 $I_2$g/100g, 112.32 $I_2$g/100g and 113.43 $I_2$g/100g respectively showing a continuous increase from $T_2$ to $T_5$ with the more and more addition of natural antioxidants.

The results of the study are also in accordance with the findings of the Nour *et al.* (2018) who studied the effect of dry tomato waste on iodine value of different edible oils.

**Table 6: Treatment mean for Iodine value (g$I_2$/100g of oil) of sunflower oil samples treated with pea pod extract**

| Treatment | Storage Days | | | | | Mean |
|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | |
| $T_0$ | 104.34±1.74 | 103.04±1.86 | 102.17±1.82 | 101.08±1.77 | 100.32±1.94 | 102.19$^e$ |
| $T_1$ | 114.44±1.94 | 113.23±2.67 | 112.36±2.62 | 111.54±2.13 | 109.66±2.42 | 112.24$^b$ |
| $T_2$ | 106.73±2.43 | 105.91±2.13 | 104.40±2.32 | 103.91±2.27 | 101.88±2.36 | 104.56$^d$ |
| $T_3$ | 111.36±1.89 | 110.74±2.54 | 110.10±2.28 | 109.13±1.98 | 107.03±2.43 | 109.67$^c$ |
| $T_4$ | 117.09±2.65 | 116.11±2.34 | 115.50±2.87 | 114.34±2.78 | 112.32±2.19 | 115.07$^a$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| **$T_5$** | 118.10±2.52 | 117.57±2.81 | 116.07±2.54 | 115.22±2.94 | 113.43±2.91 | 116.08[a] |
| **Mean** | 112.01[a] | 111.10[ab] | 110.10[bc] | 109.20[c] | 107.44[d] | |

**Refractive index**

Different factor that affect the refractive index of fat and oil is molecular weight, number of unsaturated bonds and length of chain. How much fats and oils have hardness are represented by refractive index. The outcomes of the study were manifested that there was no significant increase in refractive index of SFO-control, SFO-BHT, SFO-600, SFO-800, SFO-1000 and SFO-1200 during storage for 60 days. Outcomes of the study documented that the values of refractive index for different treatments ranges in the radius of 1.469 to 1.479 for all samples at 40°C. Fakhir and Qadir (2011) observed that refractive index value for vegetable oil was in the range of 1.468 to 1.480. Perveen *et al.* (2014) documented that refractive index ranges from to 1.459 to 1.468 for all samples at 40°C.

**Table 7: Treatment mean for Refractive index of sunflower oil samples treated with pea pod extract**

| Treatment | Storage (Days) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | |
| **$T_0$** | 1.469±0.004 | 1.470±0.002 | 1.471±0.001 | 1.472±0.002 | 1.473±0.003 | 1.471[a] |
| **$T_1$** | 1.470±0.001 | 1.471±0.003 | 1.472±0.003 | 1.473±0.004 | 1.474±0.005 | 1.472[a] |
| **$T_2$** | 1.471±0.005 | 1.472±0.001 | 1.473±0.005 | 1.474±0.002 | 1.475±0.004 | 1.473[a] |
| **$T_3$** | 1.473±0.002 | 1.474±0.004 | 1.475±0.002 | 1.476±0.003 | 1.477±0.001 | 1.475[a] |
| **$T_4$** | 1.474±0.007 | 1.475±0.006 | 1.476±0.007 | 1.477±0.006 | 1.478±0.005 | 1.476[a] |
| **$T_5$** | 1.475±0.006 | 1.476±0.007 | 1.477±0.005 | 1.478±0.005 | 1.479±0.009 | 1.477[a] |
| **Mean** | 1.472[a] | 1.473[a] | 1.474[a] | 1.475[a] | 1.476[a] | |

**Smoke Point**

During the 60 days of storage study smoke point was analyzed in sunflower oil samples at regular intervals. Lowest smoke point on the 0th day was recorded for $T_0$ (221.1) as it contain no antioxidant and $T_1$ having synthetic antioxidant had smoke point (231.5) and the smoke point observed in $T_2$, $T_3$, $T_4$ and $T_5$ were 225.3, 228.7, 234.9 and 237.7 respectively showing a continuous increase from $T_2$ to $T_5$ with the addition of natural antioxidants. The results of the study are also in accordance with the findings of the Ramroudi *et al.* (2022) who studied the physicochemical properties of oil blended with sesame oil.

**Table 8: Treatment mean for Smoke point (°C) of sunflower oil samples treated with pea pod extract**

| Treatment | Storage (Days) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | |
| **$T_0$** | 221.1±3.09 | 220.7±3.72 | 219.8±3.73 | 218.0±4.17 | 217.2±4.07 | 219.4[f] |
| **$T_1$** | 231.5±3.54 | 230.6±3.30 | 229.6±4.21 | 228.8±3.15 | 227.8±4.19 | 229.7[c] |
| **$T_2$** | 225.3±3.43 | 224.8±3.48 | 223.9±3.17 | 222.7±3.44 | 221.8±3.99 | 223.7[e] |
| **$T_3$** | 228.7±3.12 | 227.5±4.19 | 226.7±4.10 | 225.8±4.11 | 224.6±3.78 | 226.7[d] |
| **$T_4$** | 234.9±4.16 | 233.8±4.51 | 232.8±3.43 | 231.5±3.68 | 230.7±4.20 | 232.7[b] |
| **$T_5$** | 237.7±4.97 | 236.6±4.70 | 235.6±3.94 | 234.5±4.67 | 233.6±3.83 | 235.6[a] |

| Mean | 229.8$^{a}$ | 229.1$^{ab}$ | 228.1$^{b}$ | 226.9$^{c}$ | 225.9$^{c}$ | |
|---|---|---|---|---|---|---|

**Specific Gravity**

ANOVA indicated the non-significant impact of different treatments on the specific gravity of the oil. Results further revealed that storage days also have non-significant impact on the specific gravity of the sunflower oil. Combined effect of treatment and storage days also revealed the non-significant impact on the specific gravity values of the sunflower oil.

Results of the study showed that there is no significant impact among the mean values of all treatments. Lowest specific gravity on the 0$^{th}$ day was recorded for $T_0$ (0.878) and highest specific gravity on 0$^{th}$ day was recorded for $T_5$ (0.900) while $T_1$ (0.880), $T_2$ (0.881), $T_3$ (0.884) and $T_4$ (0.886) showed specific gravity value respectively. At 60$^{th}$ day the highest value of specific gravity was seen at $T_5$ (0.924) and lowest value was of $T_0$ (0.898).

**Table 9: Treatment mean for Specific gravity (g/cm$^3$) of sunflower oil samples treated with pea pod extract**

| Treatment | Storage (Days) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | |
| $T_0$ | 0.878±0.002 | 0.884±0.004 | 0.888±0.001 | 0.894±0.003 | 0.898±0.006 | 0.888$^{a}$ |
| $T_1$ | 0.880±0.004 | 0.881±0.001 | 0.897±0.004 | 0.902±0.007 | 0.909±0.003 | 0.894$^{a}$ |
| $T_2$ | 0.881±0.001 | 0.882±0.002 | 0.898±0.003 | 0.903±0.006 | 0.910±0.001 | 0.895$^{a}$ |
| $T_3$ | 0.884±0.003 | 0.891±0.003 | 0.900±0.002 | 0.904±0.002 | 0.911±0.002 | 0.897$^{a}$ |
| $T_4$ | 0.886±0.006 | 0.887±0.005 | 0.900±0.007 | 0.908±0.005 | 0.915±0.005 | 0.899$^{a}$ |
| $T_5$ | 0.900±0.005 | 0.914±0.007 | 0.917±0.006 | 0.920±0.004 | 0.924±0.009 | 0.915$^{a}$ |
| Mean | 0.884$^{a}$ | 0.889$^{a}$ | 0.900$^{a}$ | 0.905$^{a}$ | 0.911$^{a}$ | |

**Saponification value**

Saponification value indicates the presence of free fatty acids in the oil. At 0$^{th}$ day, $T_0$ showed the highest saponification value (195.91mg KOH/g) as it contains no antioxidant and $T_1$ having synthetic antioxidant showed saponification value (192.40 mg KOH/g) and saponification values observed in $T_2$, $T_3$, $T_4$ and $T_5$ were 194.92 mg KOH/g, 193.72 mg KOH/g, 192.24 mg KOH/g, 191.55 mg KOH/g respectively showing a continuous decrease from $T_2$ to $T_5$ with the addition of natural antioxidants. However, storage study showed higher value at 60$^{th}$ day for $T_0$ (207.82 mg KOH/g) and $T_5$ showed the lowest saponification value 193.76 mg KOH/g. Results further elaborated that with the increase in pea pod extract percentage in the sunflower oil, saponification values. On the other hand, with the increase in storage days, saponification values of the sunflower oil increased due to the breakdown of triglycerides into smaller, more easily saponifiable molecules such as free fatty acids through oxidation and hydrolysis.

The results were also compared with the findings of the Kehili *et al.* (2018) who investigated the oxidative stability of the sunflower and olive oils after supplementation with tomato peel.

**Table 10: Treatment mean for Saponification value (mg KOH/g) of sunflower oil samples treated with pea pod extract**

| Treatment | Storage (Days) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **$T_0$** | 195.91±1.60 | 198.61±1.84 | 200.40±2.19 | 203.48±2.95 | 207.82±2.98 | 201.08$^{a}$ |
| **$T_1$** | 192.40±1.86 | 193.62±2.43 | 195.82±2.27 | 196.01±1.82 | 197.77±1.80 | 195.13$^{c}$ |
| **$T_2$** | 194.92±2.69 | 195.36±2.34 | 199.67±2.78 | 202.99±2.70 | 205.89±2.64 | 199.77$^{ab}$ |
| **$T_3$** | 193.72±2.25 | 194.65±2.78 | 197.02±2.56 | 199.95±2.55 | 203.20±2.23 | 197.71$^{b}$ |
| **$T_4$** | 192.24±2.54 | 192.72±1.97 | 192.92±1.94 | 193.46±1.79 | 193.86±1.82 | 193.10$^{cd}$ |
| **$T_5$** | 191.55±1.78 | 191.62±1.89 | 191.90±2.18 | 192.55±1.84 | 193.76±1.78 | 192.28$^{d}$ |
| **Mean** | 193.36$^{d}$ | 194.39$^{cd}$ | 196.29$^{bc}$ | 198.07$^{b}$ | 200.39$^{a}$ | |

**Antioxidant activity analysis**

**DPPH radical scavenging assay**

DPPH test is done to assess the anti-oxidant potential of any extract. It contains proton that act as free radical which have specific absorption property and when it interacts with other free radical scavenger it lowers down. DPPH free radical has hydrogen atom that make it a scavenger. DPPH free radical scavenging assay was used to check the antioxidant potential of pea pod extract. Antioxidant potential of pea pod extract increased as its concentration increases.

$T_5$ that had 1200ppm of pea pod extract showed the high antioxidant potential throughout the storage time of 60 days. Results further elaborated that with the increase in pea pod extract percentage in sunflower oil, antioxidant activity increased. On the other hand with the increase in storage days, antioxidant activity of sunflower oil decreased because overtime, the antioxidant become oxidized and lose their effectiveness.

The reason for impact of antioxidants on DPPH radical scavenging is that they have hydrogen-donating ability and DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable molecule Shabbir *et al.* (2015).

**Table 11: Treatment mean for DPPH (% inhibition) of sunflower oil samples treated with pea pod extract**

| Treatment | Storage (Days) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | |
| **$T_0$** | 34.48±1.64 | 31.28±1.34 | 27.06±1.56 | 24.32±1.27 | 22.41±1.44 | 28.31$^{e}$ |
| **$T_1$** | 43.13±1.43 | 39.51±1.28 | 36.27±1.41 | 34.34±1.43 | 32.20±1.23 | 37.09$^{bc}$ |
| **$T_2$** | 42.13±1.35 | 37.54±1.31 | 33.65±1.19 | 30.42±1.41 | 28.19±1.39 | 34.38$^{d}$ |
| **$T_3$** | 42.69±1.56 | 38.33±1.33 | 34.09±1.14 | 33.23±1.29 | 30.11±1.27 | 35.57$^{cd}$ |
| **$T_4$** | 43.36±1.59 | 40.32±1.30 | 38.20±1.34 | 36.09±1.25 | 34.88±1.22 | 38.57$^{ab}$ |
| **$T_5$** | 43.52±1.51 | 40.58±1.50 | 38.56±1.44 | 36.53±1.41 | 35.62±1.35 | 38.96$^{a}$ |
| **Mean** | 41.78$^{a}$ | 37.93$^{b}$ | 34.64$^{c}$ | 32.49$^{d}$ | 30.57$^{e}$ | |

**Ferric reducing antioxidant power (FRAP) assay**

Antioxidant activity is measured by the FRAP assay. The ferrous-tripyridyl triazine complex, which is blue in color and has a maximum absorbance at 593 nm, is reduced from the ferric-tripyridyl triazine complex in the FRAP experiment at low pH. Instead of hydrogen ion transport, the mechanism of ferric reducing antioxidant power depends on electron transfer Rasmy *et al.* (2012).

These finding showed that pea pod extract contains high levels of phenolic compounds; these compounds have antioxidative effects and possessed antihydrolytic effects during storage.

**Table 12: Treatment mean for FRAP (µmol TE/mL) of sunflower oil samples treated with pea pod extract**

| Treatment | Storage (Days) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | |
| **$T_0$** | 107.55±1.83 | 107.24±1.84 | 106.01±2.56 | 105.56±1.76 | 104.64±1.85 | $106.20^{d}$ |
| **$T_1$** | 111.60±1.86 | 110.23±2.24 | 109.72±2.63 | 108.13±2.71 | 107.09±2.18 | $109.35^{abc}$ |
| **$T_2$** | 109.67±1.79 | 108.64±1.96 | 107.66±1.82 | 106.62±1.85 | 105.53±1.97 | $107.62^{cd}$ |
| **$T_3$** | 110.40±2.80 | 109.58±1.87 | 108.70±1.93 | 107.74±1.98 | 106.30±2.22 | $108.54^{bc}$ |
| **$T_4$** | 112.25±1.85 | 111.25±2.56 | 110.74±1.78 | 109.62±1.89 | 108.82±1.82 | $110.54^{ab}$ |
| **$T_5$** | 113.40±2.82 | 112.23±2.49 | 111.84±1.81 | 110.79±1.94 | 109.97±2.78 | $111.64^{a}$ |
| **Mean** | $110.81^{a}$ | $109.86^{ab}$ | $109.11^{ab}$ | $108.08^{bc}$ | $107.06^{c}$ | |

**Sensory analysis**

In food production, the sensory evaluation is a substantial-quality standard. The sensory evaluation of French fries for taste, color, texture, flavor and overall acceptability of product was assessed by a group of students and some trained panelists. In front of judges, samples were presented with proper labeling.

**Color**

A significant quality parameter that affects the acceptability of any product is color. In different treatments, mean value of color scores ranged from 7.20±0.02 to 7.56±0.01 but highest value was recorded in $T_0$ fries and lowest was observed in $T_3$ fries.

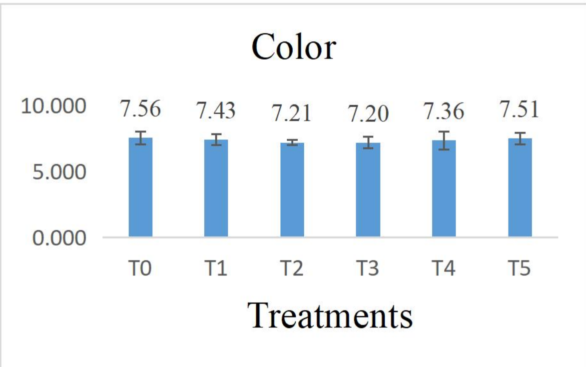


**Figure 1: Effect of treatments on color of French fries**

**Flavor**

The awareness of flavor includes many numbers of steps before substance touch and even this process continued after the substance has been swallowed.

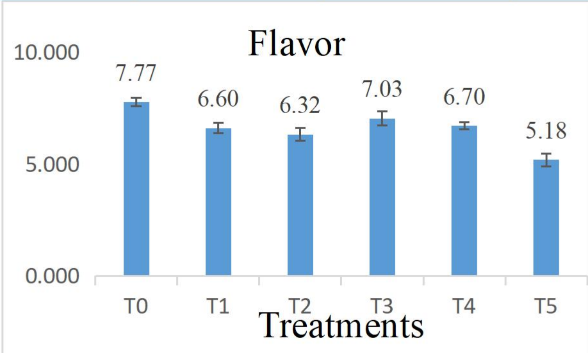


**Figure 2: Effect of treatments on flavor of French fries**

**Taste**

In the mouth, the taste is ensured by several factors such as the texture of food, the temperature of food, flavor of food and smell of food. Food can either be sweet, salty, sour, or bitter that will assess by taste buds.

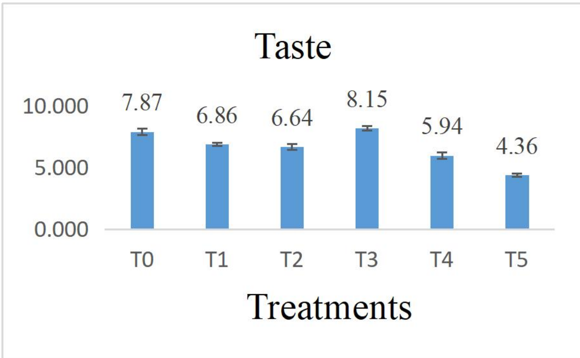


**Figure 3: Effect of treatments on taste of French fries**

**Texture**

Texture is also an important parameter in sensory evaluation. The sensory score for the texture was in the range from (7.20±0.05 to 7.49±0.02). $T_1$ gained the highest score while $T_3$ fries gained the lowest score. Scoring of texture displays that fries of $T_3$ treatment were liked by the judges while $T_1$ Moderately enjoyed by the judges.

## Conclusion

The extract was added in sunflower oil at different concentrations and storage study of 60 days at room temperature was conducted to evaluate the impact of pea pod extract on it. Sunflower oil is low in saturated fats and using natural sources for their oxidative stability surely helps in replacing synthetic additives. To check the oxidative stability of treated sunflower oil French fries was developed. Sensory evaluation showed that among all treatments $T_3$ got the highest sensory attributes and was selected as best one. Conclusively, Pea pod is a natural source of antioxidant and it can be used as functional food ingredient in different food commodities to prevent rancidity and oxidation during storage. Utilization of pea pod for extraction is beneficial.

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