Garima Gupta /J. Pharm. Sci. & Res. Vol. 12(7), 2020, 910-913

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Synergistic Effect of Antioxidant Property of Aloe Vera Leaf Skin and Mango Seed Kernel Extract on Shelf Life of Various Edible Oils

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Abstract:

Enhancing shelf life of various food products has been a priority for all the food processing industries. The improper transport and storage conditions pose the major challenge in determining the effective shelf life of any product. Edible oils and products containing fats or lipids are prone to oxidation and thus have a short shelf life. This study focuses on evaluating synergistic effect of antioxidant property of aloe vera leaf skin and mango seed kernel extract on various edible oils by reducing the lipid oxidation. At present there are many synthetic antioxidants commercially present in the market which are widely used in the edible oils. As they show a lot of side effects if used in high concentration, their limited use has been a challenge and opens up the scope for the use of natural antioxidants. The shelf life of various edible oils was estimated by adding various concentrations of ethanol and water extracts in accelerated shelf life testing (ASLT) method. Results have shown a significant reduction in peroxide values and free fatty acid values at a temperature of 20^0C and 37^0C. The ethanol extract was more effective than water extract but as this will be used for human consumption, water extract is more acceptable.

Keywords: Antioxidants, Synergistic effect,,Shelf Life, ASLT (Accelerated Shelf Life Testing), Peroxide Value, Free Fatty Acid Value

INTRODUCTION

Fats and oils are considered as valuable components of diets; and also due to an increase in development and prosperity of nations the use of oils and fats has increased in the foods. High standards for the safety and quality of foods are considered around the world. The consumers are worried about the time food will last before it starts deteriorating, food producers are worried about the long life of food products of shelves and government agencies are worried about the time till when the food will maintain its quality as listed on its label. Determination of shelf life of a food product is a difficult task but is required for the labeling of the finished product. As some products of oil degradation can be toxic in nature, thus it is important to determine the shelf life of oil for safety and quality measures. Long shelf life oils are of great importance during conditions of food shortage and natural calamities [1,2,3,4].

Shelf life of oil is mainly dependent on its susceptibility to auto oxidation which is determined by its fatty acid content. Naturally all of the vegetable oils present in the market have a significant short shelf life. Manufacturers have developed oils with fewer amounts of fatty acid and unsaturation in order to increase its stability towards oxidation[4,5,6,7,].

Accelerated Shelf Life Testing is the method of choice to determine the shelf life of food products. In order to evaluate shelf life of oils, we should first understand the definition of shelf life. Shelf life is a time period under defined storage conditions in which food product should retain its desired chemical, sensory, physical, microbiological or functional characteristics. Before analyzing the shelf life of oil, all the factors that are involved in its degradation should be considered. The actual storage studies are very time consuming for the oils which are highly stable as the take longer time to deteriorate. To increase the rate of deterioration and reduce the time needed for significant changes in quality, accelerated storage studies are performed. This helps to achieve deterioration rates early at the defined temperature. Initial readings are taken during the storage studies to deterioration rate during storage. Analytical assays performed during accelerated shelf life studies should depend upon the means of deterioration of the sample in order to be markers of oxidation [2, 8].

The primary mechanism that affects stability of packaged and processed foods is oxidative rancidity. Many factors govern the shelf life of vegetable oils, examples are temperature of storage, fatty acid content, exposure to light, oxygen concentration, presence of free-fatty acids, presence of pro-oxidants and presence of antioxidants [6, 9].

Preventing oxidative deterioration of cooking oil is a significant challenge for the food industry. Natural antioxidants are widely incorporated into foods and oils to prevent oxidation and extend shelf life. The goal of the study is to investigate the synergistic activity of antioxidants present in a combination of Aloe vera and mango seed kernel to inhibit oxidation of cooking oil. From this study, we can state that combination of Aloe vera and mango seed kernel extracts can be used as a potential natural antioxidant in food industry, extending shelf life of cooking oil. The oil samples used were as follows:

Soybean Oil:

Soybean oil appears to be dark yellow or light green in color is extracted from the soybean. Soybean oil is the most commonly used vegetable oil. While processing soybean, the oil extracted is sold as vegetable oil and the remaining soybean meal is utilized as animal feed. It is one of the major biodiesel sources in the country which constitutes around 80 percent of the domestic production.

Soybean oil constitutes four phytosterols: sitosterol, brassica sterol, stigmasterol and campestrol.

Mustard Oil:

Mustard oil extracted from mustard seeds is a fatty vegetable oil. It has a slightly pungent odor and appears dark yellow in color. Based upon the extraction method used, the mustard oils are divided into three groups. The first method involves the extraction of oil by pressing the seeds and gives fatty vegetable oil. The second method produces an essential oil which is made by first grinding the seeds then mixing them with water and finally extracting the oil by distillation. The third method includes infusing the mustard seed extract with different vegetable oils for example soybean oil. All the above-mentioned categories of oil possess a strong aroma and pungent nutty taste.

Olive Oil:

Olive oil is extracted from the fruits of olive plant. Apart from being extensively utilized in cooking all over the world, olive oil has also conquered the cosmetic industry. Based upon their method of extraction, olive oil can be classified into following groups:

1. Virgin Olive Oil **–** It is one of the most famous varieties and is extensively used in kitchen. It has a significantly low acid content and is considered good for people who are suffering from heart disease.
2. Extra Virgin Olive Oil – It is extracted by cold pressing method. In this the olive seeds are pressed under cold temperature which makes it one of the healthiest oil. Due to the complex extraction method the cost of extra virgin olive oil is very high.
3. Pure Olive Oil – It is produced by the amalgamation of virgin olive oils with refined oil. Due to its high acidic content it is not considered safe for human consumption.
4. Lampante Oil **–** This oil is mostly utilized as fuel and not considered for cooking.

Rice Bran Oil:

Rice bran oil is produced from the outer layer of the rice grain (bran) and apart from its use as cooking oil it is also used for medicine. In Japan and Asia (particularly India) Rice bran oil is commonly considered as healthy oil. Rice bran is utilized in the treating diabetes, high blood pressure, alcoholism, obesity, high cholesterol, and AIDS; for inhibiting stomach and colon cancer; for inhibiting heart and blood vessel (cardiovascular) disorder; for improving the immune system; for enhancing energy and increasing athletic capacity; for increasing liver function; and as an antioxidant. Rice bran oil can also be utilized for high cholesterol. Rice bran may help reduce cholesterol because the oil contains substances which may reduce cholesterol absorption and improve cholesterol elimination. One of the components in rice bran may reduce calcium absorption; this may help decrease the generation of some types of kidney stones.

MATERIAL AND METHOD

The shelf life of oil was determined by using accelerated shelf life testing (ASLT), where the product is stored at elevated stress conditions (storage temperature of 50^0C and 20^0C) and room temperature. Oil samples were stored using an amber bottle, and the bottles were sealed and kept in a dark room (inside an incubator) to avoid direct exposure from light. The temperatures were selected to stimulate relatively fast degradation, to determine the shelf life using ASLT (without destroying the fundamental characteristics of oil). PV and free fatty acid (FFA) concentration were measured in an accelerated storage test. All laboratory analyses were performed in triplicate, and averages are presented.

Fortified cooking oil samples were analyzed for the following parameters: (i) PV; and (ii) FFA level. These two parameters are the most common parameters to characterize oil deterioration.

ESTIMATION OF PEROXIDE VALUE

Peroxide value is a measure of the peroxides contained in the oil. The peroxides present are determined by titration against thiosulphate in the presence of KI. Starch is used as indicator. To measure PV, the AOCS Cd 8–53 method was used: 5 ± 0.5 g of oil was dissolved in 30 mL of glacial acetic acid (chloroform solution). After the addition of 0.5 mL of saturated potassium iodide with occasional shaking for 1 m and 30 mL of distilled water, the solution was titrated with Sodium thiosulphate until the yellow color faded. Starch indicator was added, and the titration was continued until the blue color disappeared. A blank determination was conducted, and the PV (mEq/kg) was calculated using the following equation:

$$\text{Peroxide value} = \frac{(S - B) \times N \text{ thiosulfate} \times 1000}{\text{weight of sample}}$$

$$= (S - B) \times N \text{ thiosulfate} \times 200$$

where: Sis the volume of Sodium thiosulphate used in the cooking oil sample until the yellow color faded (mL), Bis the volume of Sodium thiosulphate used in the blank sample until the yellow color faded (mL), *N* is the normality of Sodium thiosulphate (mEq/mL used for titration), *W* is the weight of the cooking oil sample (g).

ESTIMATION OF FREE FATTY ACIDS

To measure free fatty acid (FFA), the percentage of free fatty acid in each sample was determined by the titration method (AOCS Ca 5a-40). Ten grams of sample were weighed into a flask and then neutralized with 50 mL of 95% ethanol and 1% phenolphthalein indicator. The mixture solution was heated to a maximum of 22 °C in a steam bath for 3 min, and then 2–3 drops of 1% phenolphthalein indicator were added. The final solution was titrated against potassium hydroxide solution (0.01 N) until a permanent pink color persisted for at least 30 s. The FFA (%) was calculated using the formula as follows:

$$\text{FFAV} = (V \times N(\text{KOH}) \times 56)/W$$

where: *V* is the volume of KOH used in the blank sample until the pink color persisted (mL), *N* (KOH) is the normality of KOH, *W* is the weight of the cooking oil sample (g).

RESULTS

Peroxide Value Estimation

The Peroxide Values for four different cooking oils at three different temperatures are as follows:

Peroxide values of water extract

The result states that with reference to the sample kept at Room Temperature i.e. 37^0C, the Peroxide value remains approximately same at 20^0C and reduced drastically at 50^0C in all the oil samples. This result states that the antioxidant activity reduces with increase in temperature and thus the peroxide value, which is an indicator of lipid oxidation increases with increasing temperature.

Table 1: Peroxide values of water extract of combination of Aloe vera and Mango seed kernel extract

S. No.	Oil Sample	Temperature (^{0}C)	Peroxide value (mEq/Kg)
1	Rice Bran oil	50	12.129± 0.04
2	Rice Bran oil	37	8.241± 0.03
3	Rice Bran oil	20	8.68± 0.05
4	Mustard oil	50	30.598± 0.07
5	Mustard oil	37	20.231± 0.03
6	Mustard oil	20	21.73± 0.02
7	Soybean oil	50	6.132± 0.05
8	Soybean oil	37	4.324± 0.04
9	Soybean oil	20	4.764± 0.06
10	Olive oil	50	5.135± 0.02
11	Olive oil	37	4.002± 0.01
12	Olive oil	20	4.10± 0.03

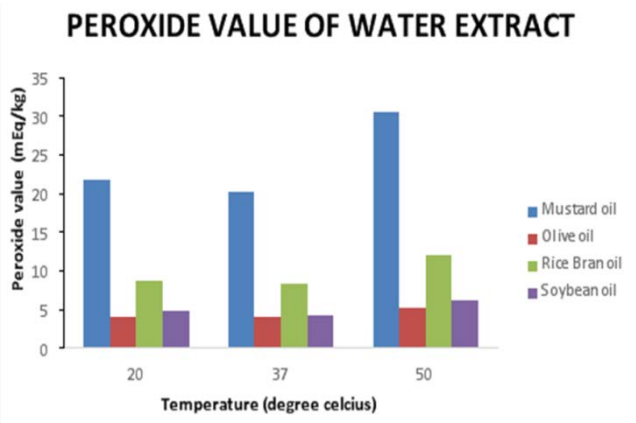

Figure 1: Peroxide values of water extract of combination of Aloe vera and Mango seed kernel extract

Peroxide values of ethanol extract

The ethanol extracts have shown better results than water extracts with reference to reduction in peroxide content. The result shows that with reference to the sample kept at Room Temperature i.e. 37^0C, the Peroxide value remains approximately same at 20^0C and reduced drastically at 50^0C in all the oil samples. This result states that the antioxidant activity reduces with increase in temperature and thus the peroxide value, which is an indicator of lipid oxidation increases with increasing temperature.

Table 2: Peroxide values of ethanol extract of combination of Aloe vera and Mango seed kernel extract

S. No.	Oil Sample	Temperature (^{0}C)	Peroxide value (mEq/Kg)
1	Rice Bran oil	50	8.279± 0.03
2	Rice Bran oil	37	3.793± 0.02
3	Rice Bran oil	20	2.589± 0.04
4	Mustard oil	50	16.49± 0.07
5	Mustard oil	37	13.12± 0.02
6	Mustard oil	20	13.79± 0.04
7	Soybean oil	50	4.458± 0.05
8	Soybean oil	37	2.391± 0.02
9	Soybean oil	20	1.923± 0.04
10	Olive oil	50	4.125± 0.03
11	Olive oil	37	3.042± 0.02
12	Olive oil	20	3.064± 0.04

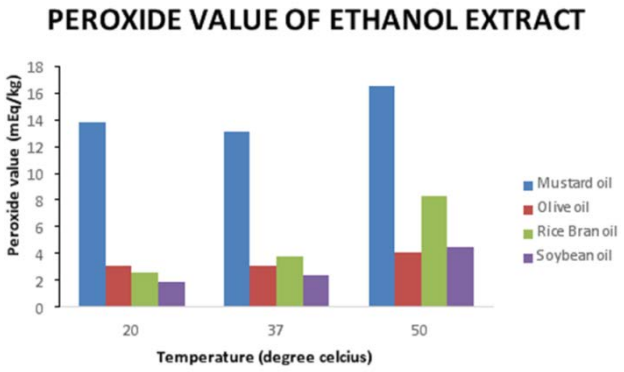

Figure 2: Peroxide values of ethanol extract of combination of Aloe vera and Mango seed kernel extract

Free Fatty Acid Estimation

Analysis of free fatty acid values of the oil samples with water extracts

The effect of combination of Aloe vera and Mango seed kernel extract on the free fatty acid content during accelerated storage conditions (20^0C, 37^0C, 50^0C) is shown in Table 3. The FFA value of oil samples with extract has shown lower values as compared to FFA values without extract. The FFA values at elevated temperatures i.e. 50^0C have shown increased values which states that the antioxidant capacity of the extract is reduced by increasing temperature as stated previously.

Analysis of free fatty acid values of the oil samples with ethanol extracts

The effect of combination of Aloe vera and Mango seed kernel ethanol extract on the free fatty acid content during accelerated storage conditions (20^0C, 37^0C, 50^0C) is shown in Table 4. The result shows that the antioxidant activity of ethanol extract is better than water extract. The FFA value of oil samples with ethanol extract has shown lower values as compared to FFA values without extract. Here also the FFA values at elevated temperatures have shown increased values which states that the antioxidant capacity of the extract is reduced by increasing temperature.

Table 3: Free fatty acid values of water extract of combination of Aloe vera and Mango seed kernel extract

S. No.	Oil Sample	FFA Value without extract (mg of KOH)	FFA Value with extract at 50^0C (mg of KOH)	FFA Value with extract at 37^0C (mg of KOH)	FFA Value with extract at 20^0C (mg of KOH)
1	Soybean Oil	1.68± 0.03	0.43± 0.05	0.14± 0.02	0.17± 0.04
2	Mustard Oil	1.90± 0.04	1.26± 0.04	0.59± 0.06	0.45± 0.05
3	Olive Oil	0.78± 0.02	0.42± 0.04	0.38± 0.07	0.40± 0.02
4	Rice Bran Oil	1.88± 0.05	1.51± 0.02	0.59± 0.05	0.54± 0.03

Table 4: Free fatty acid values of ethanol extract of combination of Aloe vera and Mango seed kernel extract

S. No.	Oil Sample	FFA Value without extract (mg of KOH)	FFA Value with extract at 50^0C (mg of KOH)	FFA Value with extract at 37^0C (mg of KOH)	FFA Value with extract at 20^0C (mg of KOH)
1	Soybean Oil	1.68± 0.06	0.37± 0.04	0.09± 0.02	0.11± 0.03
2	Mustard Oil	1.90± 0.02	0.91± 0.07	0.42± 0.04	0.43± 0.07
3	Olive Oil	0.78± 0.03	0.20± 0.05	0.12± 0.03	0.16± 0.05
4	Rice Bran Oil	1.88± 0.04	0.90± 0.03	0.40± 0.05	0.42± 0.03

Statistical Analysis

Peroxide values and Free Fatty acid assays were performed in triplicate. Mean values for different parameters were calculated and compared by analysis of variance (one-way ANOVA) using online software. Moreover, statistical differences between mean values were identified at confidence level $p<0.001$.

CONCLUSION

Oxidative rancidity is mostly determined by peroxide value estimation. It estimates the concentration of peroxides and hydro-peroxides produced in the primary stages of lipid oxidation. The two main factors that promotes the formation of peroxides are light and high temperature. The peroxides produced during oxidation are estimated by titration against thio-sulphate in the presence of potassium iodide. In this starch acts as an indicator. In this study the level of oxidation was evaluated by estimating peroxide value of different oil samples with or without combination of Aloe vera and Mango seed kernel extract two different solvents. Three different temperatures (20^0C, 37^0C, 50^0C) were selected for accelerated storage conditions studies.

The effect of antioxidant property of water extract on PV is shown in Table 1 and figure 1, whereas the effect of ethanol extract on PV is shown in Table 2 and figure 2. The studies were conducted for the storage period of 30 days. According to the results the peroxide value was increased with storage time. The peroxide value of oil sample without extract showed the maximum value. This showed their effect on lowering the peroxide formation during the lipid oxidation.

The second most frequently method used for estimating oxidative rancidity of edible oils is determination of free fatty acids produced during oxidation of lipids. The effect of combination of Aloe vera and Mango seed kernel extract water and ethanol on the free fatty acid content during accelerated storage conditions (20^0C, 37^0C, 50^0C) is shown in Table 3 and 4 respectively. After 30 days of storage the free fatty acid content of control (oil samples without extract) has increased. The oil samples with added extract have shown a reduction in free fatty acid content with reference to control. The free fatty acid content was reduced to a larger amount in samples kept at 37^0C when compared to samples kept at 50^0C. This may have occurred due to the denaturation of antioxidants compound at 50^0C.

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