International Research Journal of Multidisciplinary Scope (IRJMS), 2025; 6(1):162-170 IRJMS

Original Article | ISSN (O): 2582-631X DOI: 10.47857/irjms.2025.v06i01.02362

Synergetic Antioxidant Efficiency of *Averrhoa-carambola* Fruit's Dry Powder in Controlling Rancidity of Edible Oils

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Abstract

Averrhoa carambola is a phenolics rich fruit and shows antioxidant activity in scavenging free radicals. Milled raw soya bean and ground nut oils were used to study shelf life and amount of rancidity in the presence of *Averrhoa carambola* fruit's dry powder. The experiment was designed to record proximate parameters using a standard method of analysis. The density and viscosity of oil samples changes slightly with the amount of dry powder of the fruit. The peroxide value of experimental oil samples SBO and GNO in the presence of 0.9g of dry powder of the fruit shows a minimum value of 110 and 107meq/kg with comparison of the blank sample, 146 and 128meq/kg respectively, during the observation period of 64 days. A sample with standard antioxidant Butylated hydroxyl toluene records peroxide values of 76 and 83meq/kg, which was the least value showing an effective control of primary oxidation of edible oils. Relatively, the antioxidant property of dry powder of star fruits conspicuously controls the rancidity by retarding the peroxidation. Paraanisidine values were recorded as the lowest values for experimental oil samples, 1.5 and 1.3 for SBO and GNO respectively, with respect to 0.9g star fruit powder compared to blank oil samples *SBO and *GNO, which give 4.9 and 4.7 respectively. The rancidity of edible oils is effectively controlled by dry powder of star fruit and the fruit is available in plenty with less cost, it can be utilized as a natural preservative to increases the shelf life of edible oil.

Keywords: *Averrhoa-carambola,* Butylated Hydroxyl Toluene, Peroxide Value, Para Anisidine Value, Rancidity.

Introduction

Averrhoa-carambola (Star fruit) is a widely available fruit al around the world, conspicuously in many tropical countries like Indonesia, India, Philippines and Malaysia. It is a well-known tribal fruit in India which belongs to Oxalidaceae family. *Carambola* is a mercantile crop (1) and extensively gardening in South-east Asia and Malaysia (2, 3). It is a drought resistant evergreen tree with multipurpose uses (4). It is considered that it has taken its origin from Ceylon and the Moluccas; however, it is a tropical commercial plant (5). Star fruits are rich in nutrients with lot of health benefits and show wide biological activities like hypocholesterolemic, antioxidant, hypotensive, hypoglycemic, antitumor and immune-boosting effects (6-9). *Averrhoa-carambola* find applications in Ayurvedic and Chinese home remedies to treat fever, cough, chronic headache, diarrhea, inflammatory skin disorders (eczema), and fungal skin infections (10, 11). Ripened star fruit shows considerable antioxidant property and conspicuously removes free radical and reactive oxygen species (ROS). Researchers reported that it has appreciable amounts of flavonoids, β-carotene, proanthocyanidins, tannins, saponins, vitamin C, alkaloids, etc. It retards the efficiency of cytochrome P450 3A (12). Many reports have documented its antioxidant ability in a biological system. Shui and Leong (2004) reported polyphenolic antioxidants with the help of liquid chromatography and mass spectrometry. Many Phenolic compounds like gallic acid, L-ascorbic acid and epicatechin are identified as the main antioxidants and the star fruit residue shows maximum antioxidant activity (13). Star fruits are considered as a vital source of natural phenolic antioxidants and shows versatile health benefits (14). Researchers tested fruits and leaf extract for antioxidant properties and reported moderate effects (15, 16). The human studies by the consumption of star fruit juice give a significant

(Received 9th September 2024; Accepted 18th January 2025; Published 27th January 2025)

advance in antioxidant status (17). Natural antioxidants play a very important key role in pharmacologically active substances as they are safe and contain low toxicity, showing promised biological activities (18). Research reports indicate that the abundant natural antioxidants in star fruits and leaves can control stress due to bio-oxidation (19, 20). The free radical scavenging efficiency by DPPH was reported in the context of total antioxidant related compounds (21, 22). Researchers found that the ethanolic leaf, bark and fruit extract shows moderate to maximum antioxidant effects. The leaf gives a considerable antioxidant effect when assayed against standard with IC_{50}. The major sources of antioxidants in *A. carambola* are simple and poly-phenolics, which act as secondary metabolites and are responsible for many biological reactions and are widely distributed in roots and fruits (23-26). The versatile antioxidant properties of *A. carambola* have proven its incredible application in pharmaceutical and functional food industries. As traditional methods play a vital role compared to chemical methods in food industries during food processing, the experiment in this article was focused on the antioxidant properties of the dry fruit in controlling the rancidity of edible oils. Since star fruits are rich in phenolics, their utilization in controlling the racidity of edible oils plays a key role in the preference of natural antioxidants to synthetic ones. Thus, the raw dried powder of star fruits was used to study the antioxidant effect on controlling rancidity of soya bean and groundnut oils.

Materials and Methodology

Chemicals

Sodium-thiosulphate, potassium hydroxide, oxalic acid, glacial acetic acid, chloroform, phenolphthalein, starch and Butylated hydroxyl toluene (BHT). All the chemicals are purchased from a nice chemical supplier and are of AR and LR grade.

Sample Collection

Star fruit (*Averrhoa carambola*) were segregated from the local area in Mysore Distrct, Karnataka, India (Figure 1). Raw ground nut oil (GNO) and soya bean oil (SBO) were collected from milling directly.

Figure 1: Star fruit (*Averrhoa carambola*)

Sampling

Well ripened fruits are washed thoroughly with distilled water and shadow dried. Then the fruits are cut into small slices and ground into a fine paste using a mixture and dried in the absence of direct sunlight and then at 30°C in vacuum oven till it contains less than 5%water as shown in figure 2. The fine powder of the fruit was flushed with nitrogen and stored in an airtight brown bottle for further study.

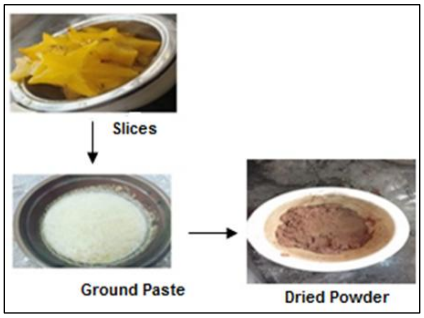

Figure 2: Different form of Star Fruit Sample

Physicochemical Analysis of GNO and SBO

Estimation of Acid Value (AV)

Filtered ground nut and soya bean oils were purchased from raw milling was used to study acid values as it is the qualitative indicator of oils. AV gives the amount of KOH required to neutralize the quantity of free fatty acid present per gram of oil. The method of determination followed was based on recommended methods of AOCS. An accurate weight of 1±0.05 gram of ground nut oil or soya bean oil was taken into a 250ml conical flask with 25ml of neutral ethanol and mixed well to dissolve. The aliquot was titrated with 0.1N KOH in the presence of phenolphthalein indicator to the end point permanent pink color. The concentration of KOH was fixed by titrating with a standard 0.1N oxalic acid solution.

$$AV = \frac{V * N * 56.1}{\text{Weight of the oil}}$$

Where, V=Volume of oil sample
N=Normality

Determination of Peroxide Value (PV)

5g of GNO or SBO was added into the erlenmeyer flask with a glass stopper containing a mixture of Acetic acid and chloroform in the ratio 3:2. 0.5ml saturated KI solution was added to the flask and allowed to evolve iodine. The evolved iodine was titrated with 0.01M sodium-thiosulphate solution using starch indicator to the equivalent point blue to colourless. The procedure was followed as per officially recommended method by AOCS.

$$PV = \frac{(S-B) * N * 1000}{\text{Weight of the sample}}$$

Where,
B=Blank titre value
S=Sample titre value
N=Normality of sodium thiosulphate solution

p-Anisidine Value (p-AV)

The amount of aldehyde and ketones formed during the storage period was estimated as per AOCS standard methods. The p-AV measures the reactivity of carbonyl bond with para anisidine amine group resulting a Schiff's base which absorbs at 350nm. 2g of GNO or SBO was dissolved in 25ml isooctane and the absorbance noted at 350nm as $A_1$with respect to isooctane (blank). 5ml of this solution and 5ml of isooctane (as blank) was taken in two separate test tubes and mixed with 1ml of para-anisidine solution (0.25 %, g/v in glacial CH_3COOH). The final solution was set for10 minutes and the absorbance determined at 350 nm noting it as A_2. The p-AV is calculated as

$$p\text{-}AV = \frac{25 * 1.2 * (A_2 - A_1)}{W}$$

Results and Discussion

Density

Density for each experimental oil sample was determined once in eight days and listed as shown in Table 1. The density of oils was measured at incubated temperature at 30°C. The value of density shown in the table linearly increases with the storage period.

Table 1: Storage time against density in the presence of variable weight ratio of star fruit powder

Oils/day		0	8	16	24	32	40	48	56	64
0.5g	GNO	0.9129	0.9130	0.9132	0.9136	0.9142	0.9148	0.9152	0.9156	0.9163
	SBO	0.9132	0.9154	0.9173	0.9184	0.9198	0.9201	0.9208	0.9214	0.9220
0.7g	GNO	0.9228	0.9232	0.9238	0.9242	0.9246	0.9253	0.9264	0.9276	0.9288
	SBO	0.9224	0.9230	0.9238	0.9242	0.9254	0.9276	0.9289	0.9304	0.9332
0.9g	GNO	0.9248	0.9256	0.9258	0.9263	0.9269	0.9276	0.9278	0.9283	0.9296
	SBO	0.9242	0.9268	0.9284	0.9324	0.9350	0.9382	0.9412	0.9468	0.9510

Figure 3 clearly indicates that the density of samples GNO and SBO showed gradual change with storage time in the presence of star fruit powder. The density of each sample changes with the weight of the dry powder of *Averrhoa carambola* during storage time. SBO shows relatively increasing density with weight ratios recording a maximum of 0.9g where GNO samples record a gradual increase in densities. The variation in densities with storage time was expected because of soluble components present in the dry powder of star fruits.

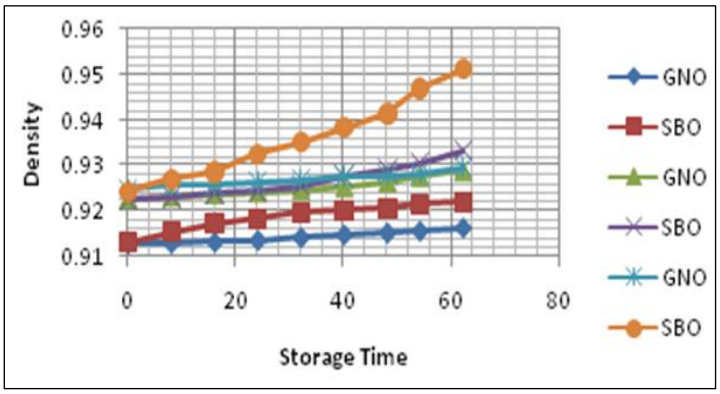

Figure 3: Plot of Density versus Storage Time of Edible Oils with Star Fruit Powder

Viscosity

Viscosity for each experimental oil sample was determined at the interval of eight days and recorded as shown in Table 2. Viscosities of the oil sample were measured using a viscometer at 35°C maintained in a thermostat.

Table 2: Storage Time against Viscosity in the Presence of Variable Weight Ratio of Star Fruit Powder

Oil/day		0	8	16	24	32	40	48	56	64
0.5	GNO	0.0574	0.0562	0.0432	0.0408	0.038	0.0354	0.0312	0.0266	0.0236
g	SFO	0.0405	0.0386	0.0304	0.0282	0.0232	0.0192	0.0148	0.0102	0.0098
0.7	GNO	0.0582	0.0574	0.044	0.0424	0.0392	0.0366	0.0324	0.0268	0.0238
g	SBO	0.0406	0.0392	0.0304	0.0296	0.0246	0.0196	0.0154	0.014	0.012
0.9	GNO	0.0593	0.0582	0.0456	0.0432	0.0396	0.0378	0.0342	0.0274	0.0242
g	SBO	0.0482	0.0396	0.0302	0.0298	0.0251	0.0202	0.0168	0.0142	0.011

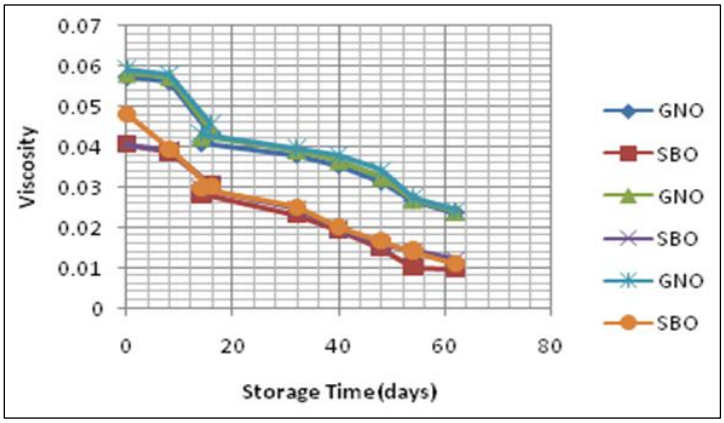

Figure 4: Plot of Viscosity versus Storage Time of Edible Oils with Star Fruit Powder

Figure 4 highlights the decreasing trends when a graph is plotted viscosity versus storage time. The decreasing trend is due to soluble compounds present in the star fruit dry powder which changes the physico-chemical properties of oil samples

Refractive Index

The refractive index for an experimental oil sample was determined after 16, 32, 48 and 64 days and recorded as shown in Table 3. All samples were maintained at an incubated temperature of 30°C and used while determinations were initiated.

Table 3: Storage time Against Refractive Index in the Presence of Variable Weight Ratio of Star Fruit Powder

Oil/day		0	16	32	48	64
0.5g	GNO	1.460	1.461	1.463	1.463	1.464
	SBO	1.466	1.466	1.467	1.468	1.469
0.7g	GNO	1.460	1.462	1.462	1.463	1.463
	SBO	1.466	1.466	1.467	1.468	1.469
0.9g	GNO	1.461	1.462	1.464	1.465	1.465
	SBO	1.467	1.468	1.469	1.469	1.470

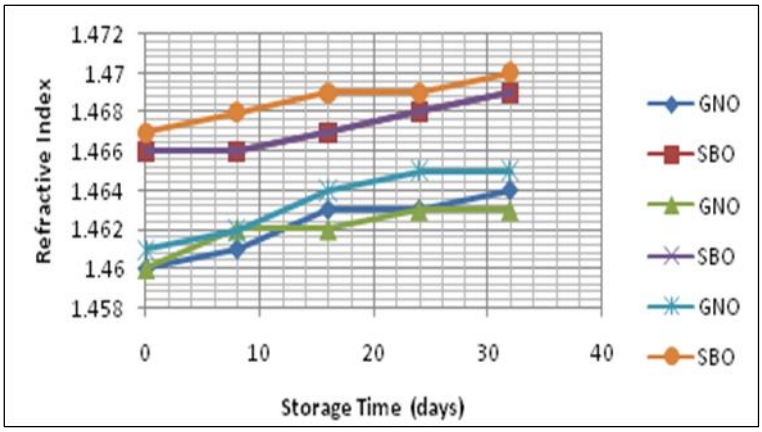

Figure 5: Refractive Index versus Storage Time of Edible Oils with Star Fruit Powder

Figure 5 clearly indicates that the refractive index of samples GNO and SBO does not change much with storage time in the presence of a star fruit sample. However, a gradual increase in refractive index was observed with storage time. This may be attributed due to simultaneous hydrolysis and peroxidation of edible oil, which has been decreased.

Acid value (AV)

The acid value for each experimental oil sample was determined at the end of 8,16,24,32 and 40 days and entered in Table 4. The samples were set for activation by adding 0.9g dry powder of star fruit along with 100mg of standard and blank.

Table 4: Storage time against AV in the Presence of Variable Weight Ratio of Star Fruit Powder

Oil/Days	0	8	16	24	32	40
SBO- Sample	1.544	1.557	1.577	1.592	1.617	1.632
SBO-Standard	1.534	1.540	1.549	1.557	1.571	1.589
SBO- Blank	1.542	1.586	1.596	1.636	1.696	1.756
GNO- Sample	2.078	2.086	2.098	2.112	2.126	2.139
GNO-Standard	2.072	2.078	2.088	2.094	2.108	2.114
GNO- Blank	2.079	2.098	2.118	2.149	2.157	2.192

Figure 6 clearly explains that the sample of SBO and GNO shows relatively lower AV compared to blank and slightly higher to that of standard. However, blank records a higher AV

1.756 and 2.192mgeq^{-1} for SBO and GNO respectively. This evidently proves that the blank undergoes fast hydrolysis to form free fatty acids which were decreased in the sample, thereby lowering the AV to 1.632 and 2.139 for SBO-sample and GNO-sample. The SBO-standard and GNO-standard record relatively lesser AV 1.589 and 2.114 respectively as it contains natural anti-oxidant TBTH. Statistically, the blank samples show 62-69 percent acid value with the standard sample to that of sample with star fruit powder 25-37 percent. The above discussion attribute considerable proof that the dry powder of star fruit control the hydrolysis of edible oils with time duration and increase the shelf life of the SBO and GNO.

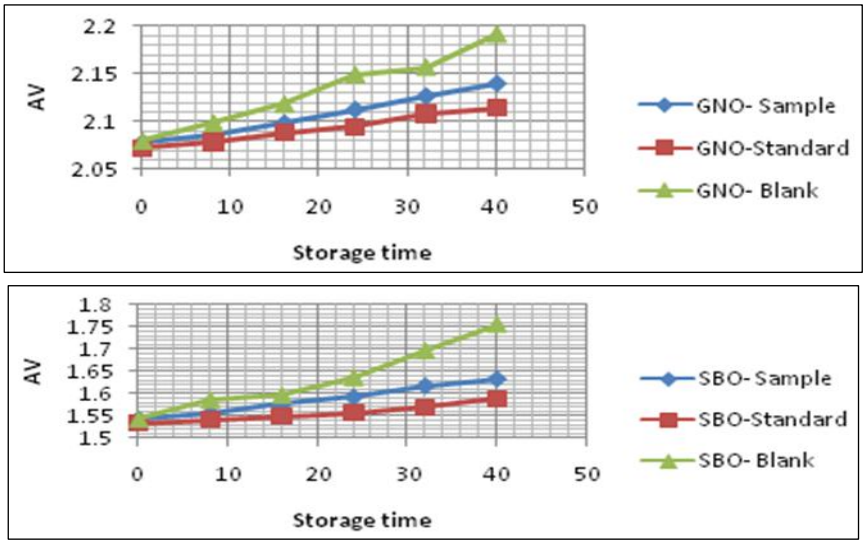

Figure 6: Plot of AV versus Storage Time of Edible Oils with Star Fruit Powder
Sample= Oil+ Star fruit dry powder, Standard = Oil+ BHT (Synthetic anti-oxidant), Blank = only oil

Peroxide Value (PV)

The PV for each experimental oil sample was determined in the sequence of eight days per trail and the values were noted as shown in Table 5. All the experimental samples were incubated at 30°C and the peroxide value of each sample was determined at the interval of eight days to forty days. Each determination was done in triplicates and taken average.

Table 5: Storage Time against PV in the Presence of Variable Weight Ratio of Star Fruit Powder

Oil/Days	0	8	16	24	32	40
SBO- Sample	36	40.12	60.2	78	90	110
SBO-Standard	36	39	45	52	63	76
SBO- Blank	38.6	54	76	93	115	146
GNO- Sample	40	50.3	65	78	91	107
GNO-Standard	40	47	52	63	71	83
GNO- Blank	41	60	74	89	101	128

A plot of peroxide versus storage time clearly highlights the antioxidant effect of dry powder of star fruits by controlling the rancidity as shown in figure 7. The lines for SBO and GNO standards record lower peroxide values compared to samples and the blank of the maximum at 110 and 107meqkg^{-1} respectively. The SBO and GNO blank show a steady increase in peroxide value and records a maximum at 146 and 128 meqkg^{-1} respectively, whereas the peroxide values of the sample lie between blank and standard having maximum at 110 and 107 meqkg^{-1}. The overall trend in the plot clearly explains that the sample controls the peroxidation of edible oils relatively to that of standards containing synthetic antioxidants. It is conspicuous that the dry powder of star fruits attributes natural antioxidant effect in controlling the rancidity of edible oils. Antioxidant efficiency is directly related to the dry powder weight ratio in the oil samples.

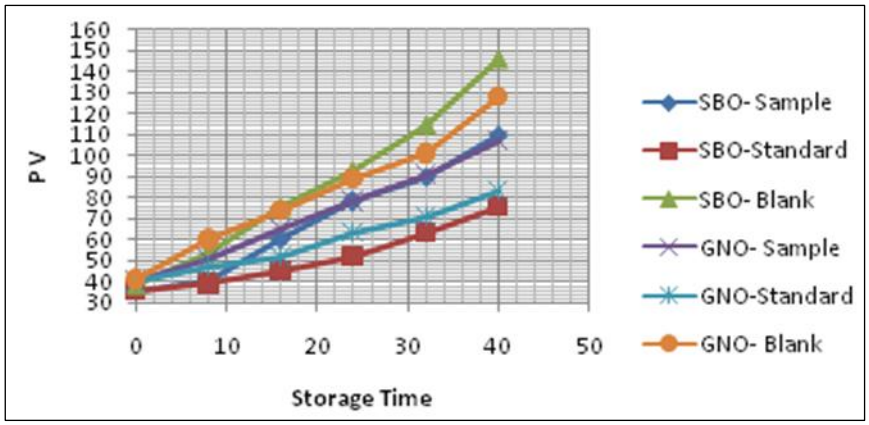

Figure 7: Plot of PV versus Storage Time of Edible Oils with Star Fruit Powder

Para-Anisidine Value (p-AV)

The p-AV for each experimental oil sample was estimated at intervals of 8 days recorded as shown in table 6. Each oil sample was incubated at 30°C and the para-anisidine value was determined at an interval of eight days, for sixty-four days with a 0.5, 0.7 and 0.9g weight ratio.

Table 6: Storage Time against p-AV in the Presence of Variable Weight Ratio of Star Fruit Powder

Oil/Days		**0g**	**0.5g**		**0.7g**		**0.9g**	
	GNO*	SBO*	GNO	SBO	GNO	SBO	GNO	SBO
0	0.2	0.2	0.2	0.3	0.3	0.1	0.2	0.1
8	0.8	0.8	0.5	0.5	0.4	0.2	0.2	0.1
16	1.2	1.1	0.7	0.7	0.6	0.4	0.3	0.2
24	1.7	1.8	1.1	0.9	0.9	0.7	0.5	0.4
32	2.2	2.1	1.6	1.1	1.2	1.0	0.8	0.6
40	2.8	2.6	2.1	1.4	1.5	1.2	1.0	0.8
48	3.4	3.6	2.4	1.7	1.8	1.6	1.1	1.0
56	4.2	4.0	2.7	2.3	2.0	1.9	1.3	1.1
64	4.9	4..7	3.1	2.5	2.2	2.1	1.5	1.3

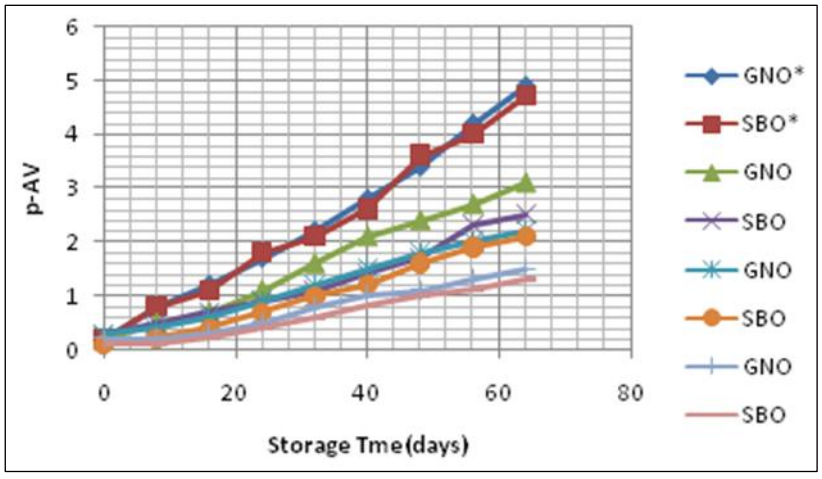

Figure 8: Plot of p-AV versus Storage Time of Edible Oils with Star Fruit Powder

The Para-anisidine value evidently increases with storage time when p-AV is plotted against storage time as shown in figure 8. The plot clearly indicates that p-AV decreases with increasing amount of star fruit and records a maximum of 1.5 and 1.3 for SBO and GNO respectively, of the sample contains 0.9g dry powder. Blank samples SBO* and GNO* recorded a maximum p-AV after 64 days, which was 4.9 and 4.7 respectively, which is relatively very high compared to the

samples. P-AV considerably decreases with weight ratio and optimum antioxidant activity was observed. The standard sample containing 100mg synthetic antioxidant records an average p-AV of ±0.5 at 64th day which is closely related with sample containing 0.9 g of dry powder of star fruit. On 64th, the blank samples record a maximum p-AV as compared to samples which indicate the drop of secondary oxidation of oils. Thus, dry star fruit powder contributes a remarkable antioxidant effect in controlling the rancidity.

Conclusion

Quality of edible oil plays the vital role in deciding hygienic food preparation and quality assurance of food products. There are many internal and external factors influencing the characteristic properties of edible oil in use. The most commonly available oils are groundnut oil, soya bean oil, coconut oil etc. Self-life is very important in the use of edible oils to a definite period as they undergo auto oxidation to hydro peroxide and secondary oxidized products like carboxiylic acids, Ketones and aldehydes. Sample and blank record higher peroxide values, 146 and 128meqkg^{-1} respectively, compared to standard SBO and GNO having maximums of 110 and 107meqkg^{-1} for 0.9g star fruit powder. The SBO and GNO blank blanks show a steady increase in peroxide value between standard and sample. The overall observation is that the plot clearly explains that the sample controls the peroxidation of edible oils relatively to that of standards containing synthetic antioxidants. It is conspicuous that the dry powder of star fruits can be used as a natural antioxidant in controlling the rancidity of edible oils. The antioxidant efficiency is directly related to the dry powder weight ratio in the oil samples. There is an increase in p-AV for SBO* and GNO* recording a maximum p-AV after 64 days, which was 4.9 and 4.7 respectively, which is relatively very high compared to the samples causing secondary oxidation of oils. The oil samples with the variable weight of star fruit powder recorded relatively lower p-AV 1.5 and 1.3 for SBO and GNO respectively, with respect to 0.9g star fruit powder.

The above records clearly explain that the dry powder of star fruit strongly controls primary and secondary oxidation of edible oils, thereby controlling the rancidity. Thus, star fruit dry powder can be used as an edible oil preservative to increase self-life and maintain the quality of oils.

Abbreviations

SBO: Sun Flower Oil, GNO-: Ground Nut Oil, DPPH: 2, 2 Diphenyl-1- picrylhydrazyl, LR: Laboratory Reagent, AR: Analytical Reagent, AV- Acid Value, PV: Peroxide Value, p-AV: Para Anisidine Value, TBTH: Ter-butyl hydroproxide, BHT: Butylated Hydroxyl Toluene, ROS: Reactive Oxygen Species, AOCS: American Oil Chemical Society.

Acknowledgement

The authors are thankful to the principal, Maharani's Science College for women, Mysore, for his cooperation in carrying out this work.

Authors Contributions

Sadashivamurthy B and Dakshayini C: Literature survey, Experiment setup and sample collections, Preeti N tallur: Experiments, data and instrumentation Pragasam A and Vinayak M Naik: Drafting of Manuscript, data interpretation, reviewing and finalizing the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Approval

The authors certify that the acceptance of publishing this manuscript anonymously declaring their consent and there is no conflict of interest.

Funding

Nil.

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