PRAJÑĀ - **Journal of Pure and Applied Sciences Vol. 24 – 25 : 190 – 194 (2017)**
ISSN 0975 - 2595

USE OF DPPH RSA TEST TO EVALUATE THE LIPID OXIDATION IN COTTON SEED OIL DURING FRYING OF FOOD PRODUCT- BESAN SEV

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ABSTRACT

This research was carried out to study the possibility of using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH RSA) test to measure the lipid oxidation events during frying. To full-fill this objective, besan sev was fried in cotton seed oil continuously for 10 hours at 180 ºC and the oil sample was collected at 2.0 hour interval. These oil samples were measured for their Peroxide value (PV), p-anisidine value (P-AV), totox value, thiobarbituric acid (TBA) test and DPPH RSA. The results showed that the decrease of the DPPH RSA was well correlated with the increase of the PV for cotton seed oil heated at 180°C (R^2 = 0.882). The DPPH RSA was negatively correlated with P-AV (R^2 = 0.869), totox value (R^2 = 0.872) and TBA test (R^2 = 0.926). From the results, it is concluded that the monitoring DPPH RSA absorbance in cotton seed oil could be useful indicator/test for measuring lipid oxidation in cotton seed oil during frying of high moisture food.

Keywords: DPPH RSA, lipid oxidation, cotton seed oil and frying

INTRODUCTION

Frying is the most popular and accepted method of cooking at home as well as at industrial level. It improves the overall sensory score of the food by increasing the flavor, crispiness and taste; moreover it also extends the self-life of food products by decreasing the moisture content during frying. During frying, oil is subjected to various chemical changes like hydrolysis, oxidation and polymerization that result in quality deterioration with respect to sensory quality and nutritive value [1]. In addition, frying also produced some unwanted compounds affecting human health. These unwanted compounds are probably nonvolatile and are produced due to oxidation and polymerization of unsaturated fatty acid [2,3].

As frying method decreases the quality of oil in respect of both food quality and human health, evaluation at lipid oxidation is very important.

At present several methods of evaluation of lipid oxidation in oil are in use. These methods are PV, Acid value, P-AV, totox value, iodine value, TBA test, conjugated dines and trines. Of all these

methods, peroxide value measures the primary oxidized product namely hydroperoxides whereas, P-AV and TBA test measures the secondary oxidation products-aldehydes. The peroxide value, P-AV and TBA test are most in use to measure lipid oxidation in oils as well as in food products.

The DPPH RSA is a test which is used to measure the total antioxidant capacity of foods of pure antioxidant compounds [4]. Recently, this method is used for the evaluation of lipid oxidation and antioxidant effectiveness of free radical scavengers in oils [5]. Yeo et al, [6] and Van et al, [7] also used DPPH radical method to measure lipid oxidation in thermally-oxidized oil.

The utilization of cotton seed oil in human consumption received attention in our country in meeting the shortage of edible oil [8]. Cotton seed oil is most popular cooking oil in the Gujarat state at house hold level as well as industrial level considering its cost compared to ground nut oil. In India, potato chips, besan sev in different sizes and taste are the two most fried food products gain industrial importance. Considering above the objective of the present study was to test the DPPH RSA as a simple test for evaluation of lipid oxidation in cotton seed oil used for frying of besan sev.

MATERIALS AND METHODS

Sample preparation and treatment: 3.0 kg of gram flour or besan (Uttam brand) was purchased from the local market of vallabh Vidyanagar-Gujarat. It was sieved in big steel plate and to this 1650 ml water and containing 90.0 gm of salt was added and made fine soft dough. The hand machine for sev making was

filled with the dough. At the same time 10.0 kg of cottonseed oil was filled up in thermostat control fryer and was switched on to raise the temperature at 180 ºC. Once temperature obtained at 180 ºC, frying of besan sev was started. One batch of besan sev was fried for 6 minutes. A total 10 batches were fried at every one hour and frying was carried out up to 10 hours. During frying, at every two hours, oil samples were collected, cooled and were stored at -20 ºC till the analysis.

Determination of DPPH RSA: The DPPH RSA was determined by the method given by Brand et al, [4]. As oil does not dissolve completely in methanol, butanol was used instead.

Physicochemical analysis: Duplicate determinations of the peroxide value [9], *p*-anisidine value [10], Thiobarbituric acid value [11] of oil samples were done. Totox value was calculated using the standard formula.

Statistical analysis: The results from the assays of oxidized sample were analyzed statistically by analysis of variance (ANOVA) using Duncan's multiple range test and Pearson's correlation using SPSS 20 software program. A p value <0.05 was considered as significant.

RESULTS AND DISCUSSION

Peroxide value is conventionally used as a measure of oxidative deterioration of oil, fat and fatty foods. It is usually presented in milli equivalent of oxygen per kg of fat [9]. It measures the peroxides formed during oxidation of fat. Peroxides are important intermediates of oxidative reactions in oils and fats because they decompose under the influence of transition metals, irradiation and elevated temperature to form free radicals. The classical method for quantification of hydroperoxides thus formed is the determination of PV. The results for DPPH RSA and PV of cotton seed oil heated at 180°C are shown in figure 1. The mean peroxide value of cotton seed oil increased significantly ($p<0.05$) in response to different frying times. It was increased about three times at the end of 10 h of frying period.

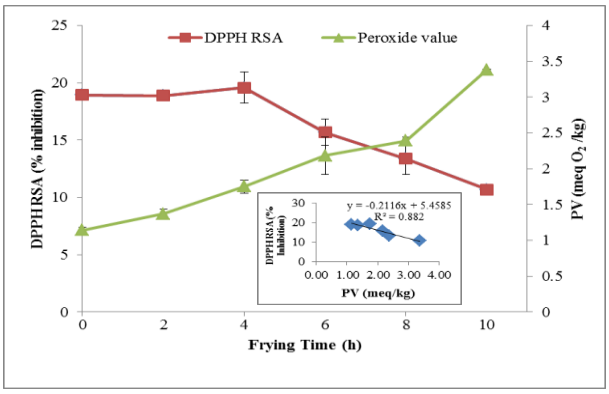

Figure 1: The PV and DPPH RSA of cotton seed oil heated at 180 °C during frying of besan sev. The relationship between PV and DPPH RSA is inserted.

Similar types of results are reported by various researchers. The increase in PV suggested that free radicals are accepted by PUFA present in cotton seed oil and lead to formation of hydroperoxide. The relationship between PV and DPPH RSA is shown in figure 1. The figure demonstrated that the DPPH RSA decreased at around the same time as primary oxidation products were formed. A reduction of DPPH RSA with the regression equation of $Y= -0.2116x+ 5.4585$ ($R^2 = 0.882$) with the PV. The PV was increased constantly till the 8h of frying time and was increased sharply between 8 to 10 h of frying. The similar types of results were reported in soybean oil at 110 °C by Van et al, [7]. The reduction in DPPH RSA could be due to the loss in naturally occurring antioxidants like phenolic compounds and α-tocopherol present in cotton seed oil. Andrikopoulos et al, [12] reported that the retention of α and (β + γ) - tocopherols was decrease as the frying period increased in virgin olive oil. They reported the complete loss of (β + γ) - tocopherols after sixth frying of potato chips in sunflower oil. They also reported the deterioration of phenolic compounds during the frying. Tannic acid, oleuropein and hydroxytyrosol-elenolic acid dialdeydic form showed remarkable resistance in all frying sessions in both frying methods (deep frying and pan frying), while hydroxytyrosol and hydroxytyrosol-elenolic acid were eliminated faster.

When hydroperoxides break down, they produce volatile alhehydes like hexanal, leaving behind a non-volatile portion of the fatty acid that remains as a part of the

glyceride molecule. This non-volatile reaction product can be measured by reaction with anisidine. High anisidine values are an indication that a fat has been oxidized even when TBA and other aldehydes tests give low results because volatile aldehydes, may incidentally or intentionally be removed during processing. Anisidine value is defined as 100 times the absorbance (at 350 mm) of a solution resulting from reaction of 1 g of fat in 100 ml of solvent.

The mean values of P-AV of cotton seed oil at varying frying time interval and its relation with DPPH RSA is shown in figure 2, The P-AV was 33.95 of fresh oil and was increased significantly ($p<0.05$) about approximately 4 times at the end of 10 h of frying time. The increased P-AV during frying was reported by Lee et al, [13] in palm oil and soybean oil. The DPPH RSA value decreased at the same time when secondary oxidized products were formed. A reduction of DPPH RSA was seen with the regression equation of $Y= -8.6215x+ 212.91$ with $R^2= 0.869$. There was a sharp increase in P-AV after two hours of frying and there after increase steadily till the 10 h of frying period. The DPPH RSA was reduced sharply after four hours of frying. These results revealed that the cotton seed oil contain enough antioxidants which can neutralized the free radicals produced in response of frying of high moisture food that is besan sev in oil up to four hour of frying.

Hydroperoxides are only transitory intermediates and decompose into various carbonyl and other compounds. Although a properly conducted peroxide value determination is a good guide to fat quality, oxidized oil can be reprocessed to give a deceptively low peroxide value. In such cases, the presence of secondary products plus lower level of antioxidant present will enable further rapid oxidation to occur. For this reason it is preferable not to rely solely upon peroxide values as an index of oil quality, but undertake an anisidine test in addition, such as "Totox" determination is very useful in the detection of oxidation of lipid. An expression termed the totox or oxidation value (OV), which is equivalent to 2x peroxide value + anisidine value, has been suggested for the assessment of oxidation in oils.

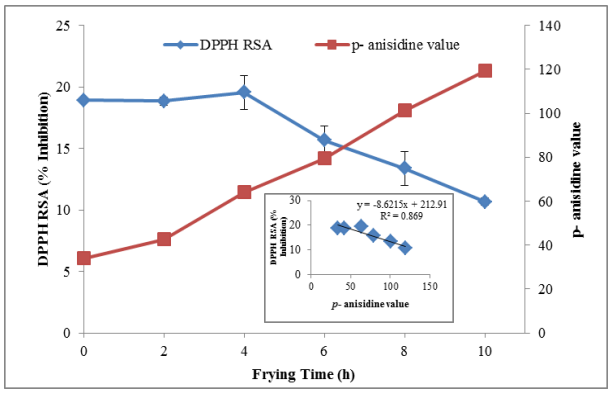

Figure 2: The P-AV and DPPH RSA of cotton seed oil heated at 180 °C during frying of besan sev.

The mean values of totox value of cotton seed oil at varying frying time interval and its relation with DPPH RSA is shown in figure 3, the totox value of fresh oil was 36.24 and it increased significantly to 126.16 after 10 hours of frying. The relationship between DPPH RSA and totox value is expressed in figure 3. This relation suggested the increase of totox value and decrease in DPPH RSA after 4 h of frying of besan sev in cotton seed oil at 180 ˚C. Van et al, [7] reported that the anti radical power of oil with intermediate frying decreased significantly ($p<0.05$) slower than oil that was heated alone. They explained their results by the fact that during frying water in the form of steam was released from the product. The contact of steam with oil induced hydrolysis, when a steam blanket covered the oil, so that the oxygen supply was limited [14].

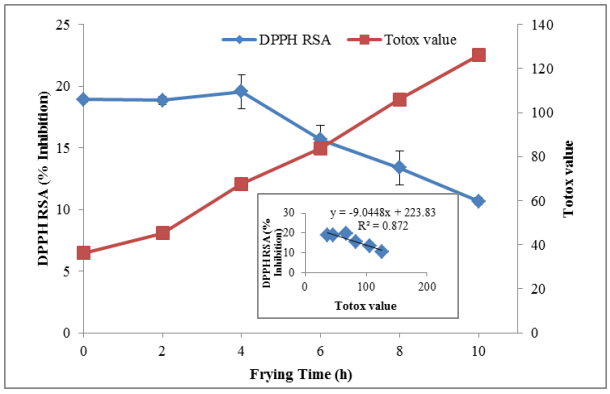

Figure 3: The totox value and DPPH RSA of cotton seed oil heated at 180 °C during frying of besan sev.

There are two stages of oil oxidation, i.e. the first phase is the formation of hydroperoxides and the second one is the decomposition of hydroperoxides to produce

secondary oxidation products, which could react with TBA reagent to produce colored compounds that absorb usually at 530 nm.

The mean values of TBA of cotton seed oil at varying frying time interval and its relation with DPPHRSA is shown in figure 4, The TBA was 3.00 µg/gm of oil of fresh oil and was increased significantly ($p<0.05$) about approximately three times at the end of 10 h of frying time. Regression between DPPH RSA and TBA showed positive relationship (R^2= 0.926).

Table 1: Pearson's correlation between PV, P-AV, totox value, TBA and DPPH RSA of cotton seed oil heated at 180 °C

	Peroxide value	*p*- anisidine value	totox value	TBA
***p*- anisidine value**	0.973**			
totox value	0.975**	1.000**		
TBA	0.974**	0.990**	0.990**	
DPPH RSA	-0.938**	-0.932**	-0.934**	-0.962**

**. Correlation is significant at the 0.01 level (2-tailed).

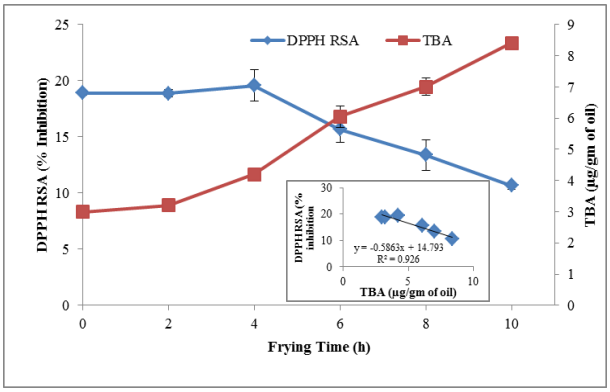

Figure 4: The TBA and DPPH RSA of cotton seed oil heated at 180 °C during frying of besan sev.

In this study the correlation between various conventional methods (PV, P-AV, totox, TBA and DPPH RSA) was studied and results obtain are presented in table 1. DPPH RSA correlated negatively with PV (R^2 = -0.938, $p< 0.01$), P-AV (R^2 = -0.932, $p< 0.01$), totox value (R^2 = -0.934, $p< 0.01$) and TBA (R^2 = -0.962, $p< 0.01$). Sikwese and Duodu [15] reported that sunflower oil with crude phenolic extract (CPE) had lower peroxide values compared to control oils.

They also reported that sunflower oil with tertiary butyl hydroquinone (TBHQ) had lower peroxide value.

These results suggested that the DPPH RSA has shown a strong relation with all four conventional methods commonly used for evaluation of oxidation status in fat or oil exposed to heat alone or during frying of various foods, hence from these results it is recommended that the DPPH RSA could also be used as one of the methods of measuring lipid oxidation in oil or fat or high fat containing foods. It is also a simple and less time consuming method.

ACKNOWLEDGMENT:

Author is thankful to the University Grant Commission, New Delhi, for financial support under Major Research Project (F.31-277/2005(SR); Date:5/4/2006).

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