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Effect on peroxide and thiobarbituric acid Value of Cow Ghee Spiked with Vegetable oils and Synthetic Antioxidants during Storage

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Abstract
Ghee is an expensive dairy product and therefore, has remained a target of adulteration with low priced fats. Ghee available in the market may be adulterated with different kind of vegetable oils/synthetic antioxidants. In the present study, investigation was made to observe the chemical changes like peroxide value and thiobarbituric acid (TBA) value that takes place in ghee after being spiked with vegetable oils (Palm oil and Sunflower oil) and synthetic antioxidants (Butylated Hydroxyanisol and Butylhydroquinone) during storage at ambient temperature (30°C). The peroxide and **TBA** values of all the samples were increased significantly at room temperature in the following order, PO >SFO> PO spiked PG> SFO spiked PG>PG>BHA spiked PG> TBHQ spiked PG and SFO> SFO spiked PG> PG> PO spiked PG> PO>BHA spiked PG> TBHQ spiked PG, respectively.

Keywords: Peroxide value, TBA value, Storage, cow ghee, vegetable oils, Synthetic antioxidant

1. Introduction
Ghee standard states that no synthetic antioxidant is permitted in ghee. On the contrary in vegetable oils the synthetic antioxidants like Butylated Hydroxyanisol (BHA), Tertiary Butylhydroquinone (TBHQ) are permissible to the tune of maximum 200 ppm (FSSR, 2011).
Milk fat's adulteration has usually been a severe problem in the Indian Dairy Industry. Unscrupulous traders for the unethical economic gains do this malpractice of partly replacing high priced fats with low priced oils (e.g., Palm oil, sunflower oil etc.). The common tests performed to check such adulteration include the physcio- chemical parameters specified by Food Safety and Standards Authority of India in its ghee standard (FSSAR, 2011). In addition to these tests some new tests has also been adopted by the Industry as their internal quality check like S- values as defined for cow milk fat by ISO/IDF (ISO, 2019). Similarly, some physical and chemical methods such as; melting point, iodine value, saponification number, percentage of TSC, saturated and unsaturated fatty acids reported to be e useful to determine the adulterated of milk fat with different ratios of coconut oil (Salem *et al.*, 2019). However, the rapid / platform tests to detect the added vegetable oils in ghee are lacking. In the present study the attempt has been made to see the changes in the peroxide and thiobarbituric acid Value of ghee samples adulterated with vegetable oils as well as synthetic antioxidants and draw a conclusion, whether these tests are useful in stored ghee samples.

2. Materials
2.1 Chemicals
Trichloroacetic acid($C_2HCl_3O_2$) was purchased from Sisco Research Laboratory Pvt. Ltd, Maharashtra, India; Hydrochloric acid (HCl) was purchased from Thermo Fisher Scientific, India Pvt. Ltd; TBA: Thio Barbituric acid ($C_4H_4N_2O_2S$), Anhydrous sodium sulphate (Na_2SO_4), Glacial Acetic acid (CH_3COOH), Iodate free Potassium iodide (KI), Potassium iodate (KIO_3), Sodium Carbonate (Na_2CO_3), sodium thiosulphate($Na_2S_2O_3$), AR grade; Sodium sulphate anhydrous (Na_2SO_4) were purchased from Titan Biotech Ltd Bhiwadi, India.

2.2 Solvents
Chloroform (AR grade), Ethanol absolute and Acetonitrile (HPLC grade) were purchased from S.D Fine- chem. Ltd. Mumbai, India; Petroleum Ether (AR grade) was purchased from Glaxo Laboratories India Ltd., Bombay;

2.3 Synthetic antioxidants
Butylated Hydroxyanisol (BHA), Butylhydroquinone (TBHQ) and Ascorbic acid were purchased from Sigma Aldrich, St. Louis, USA;

2.4 Evaluation of oxidation in stored adulterated ghee samples: Control and ghee samples spiked with antioxidants (BHA & TBHQ) and vegetable oils (Palm oil and Sunflower oil) were stored in HDPE plastic bottles at 30ºC. The above samples were evaluated for their status of oxidation by (i) Peroxide value and (ii) TBA value at every 15 days interval up to 9 months. The methods are as follows:

2.5 Determination of Peroxide value of ghee
The peroxide value (PV) of ghee was determined by iodometric method, as described in IS: 3508:1966; reaffirmed in 2018.

2.6 Determination of Thio Barbituric acid (TBA) value of ghee: TBA value of ghee samples was determined by the method, as described in Asha *et al.* (2015) [2].

2.7 Statistical analysis
The data obtained in the present study was subjected to one way and two-way Analysis of variance (ANOVA) for the significant difference in the means via Duncan's Multiple Comparison test performed at 95% confidence interval with IBM SPSS Statistics (version 20). The results were expressed as mean with standard deviation. The effect of storage days treatments and their combined effect on peroxide value, thiobarbituric acid value of ghee were analysed using factorial design of SPSS.

3. Result and Discussion
3.1 Effect of storage period on the peroxide value
Peroxide value test measures the primary oxidation products of lipid oxidation. Peroxide value expressed as mM of active oxygen/kg of ghee. Therefore, the stored samples were evaluated for their status of oxidation by peroxide value at every 15 days interval up to 9 months.

3.2 Effect of storage period on the peroxide value of pure cow ghee (PG), palm oil (PO) and PO spiked PG samples at ambient temperature (30 ºC)
Peroxide value was determined and expressed as mM of active oxygen/kg of ghee. These results have been depicted in Table 1.0. It is evident from the data that peroxide value of PG remained unaltered till 60 days of storage at ambient temperature. On the contrary, in case of ghee samples spiked with PO @ 5, 7.5 and 10%, peroxide value remained unaltered till 15 days and on 30th day of analysis it showed an increase. However, the peroxide value of ghee samples spiked with PO @ 2.5% and 1.0% the increase in the peroxide value was observed at 45 and 60 days of storage, respectively. Palm oil had the peroxide value (19.73±0.27) on zero day of storage. It was also observed that in spiked ghee samples, the peroxide value significantly increased ($P<0.05$) as the storage period progressed and observed increase was in the order of S1> S2> S3> S4>S5 (28.80±0.53, 26.80±0.71, 25.33±1.33, 23.73±0.71 and 22.13±0.27), respectively. This increase was corresponding to the level of added PO in the ghee. The peroxide value of control PG significantly increased ($P<0.05$) from 0 on 0 days and as the storage period progressed beyond 60 days, it reached to 20.40±0.71 after 9 months (270 days), which was close to the 0-day value of palm oil.
The result of ANOVA (Table 9.0) showed that the treatment i.e., palm oil spiked ghee samples had significant effect on the peroxide value. Similarly, storage days had also significant effect on the peroxide value. The interaction effect between treatment and storage days on the development of peroxide value was statistically significant at 95% level of confidence with *p*- value <0.001. The development of higher peroxide value in PO and PO spiked ghee may be attributed to the presence of higher amount of poly, mono and di-unsaturated fatty acids in palm oil.
The findings of present study are in accordance with the findings of Chaudhari *et al.*, (2016). They reported that the peroxide value of cow ghee and palm oil spiked cow ghee @20% were 0.00-3.92 and 0.00-11.26) mM O_2/kg of ghee, respectively during 0-15 days of storage at 80 ºC.
Gosewade *et al.* (2016) [6] also reported that the peroxide value of cow ghee and buffalo ghee increased from (0.00-2.59) and (0.00-3.19) mM O_2/kg of ghee, respectively during 0-10 days of storage at 80 ºC. Another study also reported that the peroxide value of cow ghee was increased from 0.00-29.65 mM O_2/kg of ghee on storage from 1- 210 days at 37±1ºC and 0.00-24.89 mM O_2/kg of ghee during storage from 1-60 days at 60±1 ºC (Rahila, 2016) [9].
Almeida *et al.* (2016) [1] also reported that the peroxide value of refined palm oil was increased significantly from (00.18-58.36) meq O_2/kg of oil from 1-12 months of storage at 26-32 ºC.

Table 1: Effect of storage period on the peroxide value of cow ghee (PG), Palm oil (PO) and PO spiked PG samples at ambient temperature (30 ºC)

Storage Days	Peroxide Value (mM O_2/kg ghee)						
	PG	PO	S1	S2	S3	S4	S5
0	0.00^{aA}	19.73 ± 0.27^{aB}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
15	0.00^{aA}	20.27 ± 0.53^{aB}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
30	0.00^{aA}	21.27 ± 0.27^{aE}	5.33 ± 0.27^{bD}	4.40 ± 0.27^{bC}	3.20 ± 0.27^{bB}	0.00^{aA}	0.00^{aA}
45	0.00^{aA}	22.93 ± 0.53^{bE}	6.93 ± 0.53^{bcD}	5.20 ± 0.53^{bC}	4.27 ± 0.53^{bcBC}	3.73 ± 0.27^{bB}	0.00^{aA}
60	0.00^{aA}	23.07 ± 0.46^{cF}	8.53 ± 0.46^{cdE}	6.40 ± 0.53^{cD}	5.33 ± 0.46^{cC}	4.80 ± 0.46^{cBC}	3.20 ± 0.27^{bB}
75	4.27 ± 0.27^{bA}	24.53 ± 0.53^{cE}	9.60 ± 0.27^{deD}	8.00 ± 0.53^{dD}	6.93 ± 0.53^{dC}	5.87 ± 0.46^{dBC}	4.40 ± 0.46^{bAB}
90	5.87 ± 0.46^{cA}	25.60 ± 0.27^{cD}	10.67 ± 0.53^{efC}	9.20 ± 0.27^{deBC}	8.53 ± 0.46^{eB}	6.93 ± 0.53^{eA}	5.60 ± 0.71^{cA}
105	7.47 ± 0.53^{dAB}	27.73 ± 0.46^{dE}	11.73 ± 0.46^{fgD}	10.40 ± 0.53^{efCD}	9.07 ± 0.53^{efBC}	8.53 ± 0.53^{fAB}	6.80 ± 0.53^{cA}
120	9.07 ± 0.46^{eA}	29.87 ± 0.53^{eC}	12.80 ± 0.27^{ghB}	11.20 ± 0.46^{fgB}	10.13 ± 0.71^{fgA}	9.87 ± 0.27^{gA}	8.00 ± 0.27^{dA}
135	10.13 ± 0.53^{eA}	31.47 ± 0.27^{fC}	13.87 ± 0.53^{hB}	12.40 ± 0.27^{ghB}	11.20 ± 0.27^{gA}	10.93 ± 0.27^{hA}	9.60 ± 0.27^{eA}
150	11.73 ± 0.53^{fA}	32.53 ± 0.53^{fD}	15.07 ± 0.46^{iC}	13.60 ± 0.53^{hB}	12.80 ± 0.46^{hAB}	12.27 ± 0.53^{iA}	11.20 ± 0.46^{fA}
165	12.80 ± 0.27^{fgA}	34.13 ± 0.46^{gD}	16.40 ± 0.53^{jC}	14.80 ± 0.27^{iB}	13.87 ± 0.53^{hA}	13.33 ± 0.27^{jA}	12.80 ± 0.27^{fA}
180	13.87 ± 0.53^{ghA}	35.73 ± 0.27^{hD}	17.60 ± 0.53^{jC}	16.00 ± 0.71^{iB}	15.20 ± 0.46^{iB}	14.40 ± 0.53^{kA}	13.27 ± 0.53^{fA}

195	14.40±0.27^{hiA}	37.33±0.53^{iE}	18.80±0.27^{kD}	17.20±0.46^{jC}	16.40±0.53^{iB}	15.60±0.27^{lB}	14.60±0.27^{gA}
210	15.47±0.46^{ijA}	38.93±0.71^{jE}	20.40±0.53^{lD}	18.40±0.53^{kC}	18.00±0.71^{jBC}	17.07±0.53^{mAB}	16.53±0.53^{hA}
225	16.53±0.53^{jkA}	40.53±0.53^{kF}	22.00±0.27^{lmE}	20.00±0.27^{lD}	19.60±0.53^{kC}	18.40±0.53^{nB}	17.60±0.46^{hiAB}
240	17.60±0.71^{klA}	42.13±0.27^{lF}	24.00±0.53^{mnE}	21.60±0.71^{mD}	20.80±0.71^{lC}	20.27±0.53^{oB}	18.67±0.53^{ijA}
255	18.73±0.53^{lA}	43.73±0.53^{mG}	26.40±0.53^{noF}	23.60±0.53^{nE}	22.40±0.53^{mD}	21.60±0.27^{pC}	19.73±0.46^{jB}
270	20.40±0.71^{mA}	44.80±0.71^{nG}	28.80±0.53^{pF}	26.80±0.71^{oE}	25.33±1.33^{nD}	23.73±0.71^{qC}	22.13±0.27^{kB}

Values are represented as Mean ± SD, n=3; $^{a, b \& A, B}$ – different superscript row-wise and column-wise, respectively differ significantly (P<0.05)
PG: Pure Ghee; PO: Palm Oil; S1: PG spiked with PO @ 10%; S2: PG spiked with PO @ 7.5%; S3: PG spiked with PO @ 5%; S4: PG spiked with PO @ 2.5%; S5: PG spiked with PO @ 1%

3.1. 2. Effect of storage period on the peroxide value of PG, sunflower oil (SFO) and SFO spiked PG samples at ambient temperature (30 ºC)

Peroxide value was determined and expressed as mM of active oxygen/kg of ghee. These results have been depicted in Table 2.0. It is evident from the data that peroxide value of PG remained almost nil till 60 days of storage. On the contrary, in case of ghee samples spiked with SFO @ 2.5, 5 and 7.5, 10 % showed an increase in peroxide value on45, 30, and 30th day of storage. However, the peroxide value of ghee sample spiked with SFO @ 1% the increase in the peroxide value was observed at 60th day of storage. It is also evident from the data (Table 2.0) that SFO had the peroxide value (17.07±1.07) on zero day of storage. It was also observed that in spiked ghee samples, the peroxide value significantly increased (P<0.05) as the storage period progressed and increase was corresponding to the level of added SFO in the ghee. The peroxide value of control PG significantly increased (P<0.05) from 0 on 0 days to 20.40±0.71 after 9 months (270 days), whereas, peroxide value of SFO increased to 41.60±1.71 on 270 days of the storage. This increase was higher than those of SFO spiked PG samples in the order T_1, T_2, T_3, T_4 and T_5 (26.67±0.71, 24.53±0.71, 23.20±0.71, 21.87±0.53 and 20.80±0.53), respectively.

The result of ANOVA (Table 10.0) illustrated that sunflower oil spiked ghee samples had significant effect on the peroxide value. Similarly, storage days had also significant effect on the peroxide value. The interaction effect between treatment and storage days on the development of peroxide value was significant at 95% level of confidence with p- value <0.001. The higher development of peroxide value in SFO and SFO spiked ghee may be due to the higher amount of polyunsaturated fatty acids approx. (65-67%) (Aladedunye and Przybylski, 2009)

Gazwi (2017) [4] reported that the peroxide value of sunflower oil increased significantly from (8.50-65.20) meq O_2/kg of oil from 1-20 days of storage at 65 ºC.

Table 2: Effect of storage period on the peroxide value of PG, sunflower oil (SFO) and SFO spiked PG samples at ambient temperature (30 ºC)

Storage Days	Peroxide value (mM O_2/kg ghee)						
	PG	SFO	T_1	T_2	T_3	T_4	T_5
0	0.00 ^{Aa}	17.07±1.07^{aB}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
15	0.00^{aA}	17.07±1.07^{aB}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
30	0.00^{aA}	18.07±1.07^{aC}	4.53±0.27^{bB}	4.00±0.27^{bB}	0.00^{aA}	0.00^{aA}	0.00^{aA}
45	0.00^{aA}	19.20±1.00^{bF}	6.20±0.46^{cE}	5.60±0.46^{cB}	3.60±0.27^{bC}	3.20±0.27^{bB}	0.00^{aA}
60	0.00^{aA}	20.80±1.00^{bcF}	7.40±0.27^{cdE}	6.40±0.27^{dE}	4.80±0.46^{cD}	4.00±0.46^{cC}	2.93±0.27^{bB}
75	4.27±0.27^{bA}	21.87±1.53^{cdD}	8.80±0.46^{dC}	7.73±0.46^{deC}	6.13±0.27^{cB}	5.33±0.27^{dAB}	4.40±0.27^{cA}
90	5.87±0.53^{cA}	23.47±1.53^{deD}	9.40±0.27^{dC}	8.53±0.27^{eBC}	7.73±0.27^{dB}	7.73±0.27^{eB}	6.40±0.46^{dA}
105	7.47±0.46^{dA}	24.53±1.53^{eE}	10.93±0.27^{eD}	10.13±0.46^{fCD}	9.07±0.27^{eBC}	8.53±0.27^{eAB}	8.00±0.53^{eAB}
120	9.07±0.53^{eA}	26.67±1.53^{fF}	13.33±0.27^{fE}	12.00±0.27^{gDE}	10.83±0.53^{fCD}	10.67±0.27^{fBC}	9.60±0.46^{fAB}
135	10.13±0.53^{eA}	28.27±1.53^{fgF}	14.93±0.27^{gE}	13.20±0.27^{hDE}	12.40±0.27^{gCD}	12.27±0.27^{gBC}	11.20±0.46^{gAB}
150	11.73±0.46^{fA}	29.80±1.27^{gE}	15.73±0.27^{ghD}	14.40±0.46^{iD}	13.87±0.53^{gC}	13.07±0.27^{gBC}	12.27±0.27^{hAB}
165	12.80±0.53^{fgA}	30.93±1.53^{hF}	16.80±0.46^{hiE}	15.60±0.27^{ijD}	14.93±0.46^{hCD}	14.13±0.27^{hBC}	13.33±0.27^{iAB}
180	13.87±0.53^{ghA}	32.00±1.00^{hiF}	17.33±0.27^{ijE}	16.53±0.27^{jDE}	15.73±0.27^{hCD}	14.93±0.27^{hiBC}	14.20±0.46^{ijAB}
195	14.40±0.27^{hiA}	33.07±1.53^{ijE}	18.60±0.27^{jD}	17.60±0.53^{kCD}	16.80±0.46^{iC}	15.73±0.27^{iB}	14.93±0.27^{jAB}
210	15.47±0.53^{ijA}	34.67±1.53^{jkE}	20.53±0.71^{kD}	18.40±0.27^{lC}	17.87±0.27^{jBC}	17.07±0.27^{jB}	16.53±0.27^{kAB}
225	16.53±0.46^{jkA}	36.27±1.71^{klE}	22.29±0.56^{lD}	20.00±0.46^{mC}	18.93±0.27^{kBC}	18.13±0.27^{kB}	17.60±0.53^{lAB}
240	17.60±0.53^{klA}	37.87±1.53^{lmF}	23.47±0.53^{mE}	21.60±0.53^{nD}	20.53±0.46^{lCD}	19.47±0.27^{lBC}	18.67±0.46^{mAB}
255	18.73±0.53^{lA}	39.47±1.71^{mF}	24.80±0.46^{nE}	23.20±0.46^{oD}	21.60±0.53^{mC}	20.53±0.27^{mBC}	19.60±0.71^{mB}
270	20.40±0.71^{mA}	41.60±1.71^{nF}	26.67±0.71^{oE}	24.53±0.71^{pD}	23.20±0.71^{nC}	21.87±0.53^{nB}	20.80±0.53^{nAB}

Values are represented as Mean ± SD, n=3; $^{a, b \& A, B}$ – different superscript row-wise and column-wise, respectively differ significantly (P<0.05)
PG: Pure Ghee; SFO: Sunflower Oil; T_1: PG spiked with SFO @ 10%; T_2: PG spiked with SFO @ 7.5%; T_3: PG spiked with SFO @ 5%; T4: PG spiked with SFO @ 2.5%; T5: PG spiked with SFO @ 1%

3.1.3. Effect of storage period on the peroxide value of PG and TBHQ spiked PG samples at ambient temperature (30 ºC)

It is evident from the results (Table 3.0) that peroxide value remained unaltered in PG samples till 60 days of storage. On the contrary, in case of ghee samples spiked with TBHQ @ 0.0025, 0.005, 0.01 and 0.02% showed an increase in peroxide value after 105, 120, 135 and 150 days, respectively. It was observed that peroxide value in ghee samples spiked with TBHQ @ 0.02% was low as compared to the samples spiked with lower concentration of TBHQ. Increase in the peroxide value on account of storage was significant (P<0.05) and was inversely related to the level of added TBHQ in the ghee. The peroxide value of control PG significantly increased (P<0.05) from 0 on 0 days to 20.40.73±0.71 after 9 months (270 days).

The result of ANOVA (Table 11.0) illustrated that the treatment i.e., TBHQ spiking had significant effect on the

peroxide value. Days of storage also had a significant effect on peroxide value. The interaction effect between treatment and storage days on the development of peroxide value was significant at 95% level of confidence with *p*- value <0.001. The higher development in peroxide value of PG sample may be due to the more primary oxidation products formed in lack of antioxidant. Asa *et al*. (2015), also reported the same findings and reasoning for ghee added with BHA and orange peel extract. Rahila (2016) [9] also reported that the peroxide value of cow ghee added with BHA (synthetic antioxidant) was increased from 0.00 on 1st day of storage at 37±1ºC to 19.69 mM O_2/kg after 210 days. She also reported that the increase was from 0.00 on 1st day of storage at 60±1ºC to 23.02 mM O_2/kg of ghee after 60 days.

Table 3: Effect of storage period on the peroxide value of PG and antioxidant (TBHQ) spiked PG samples at ambient temperature (30 ºC)

Storage Days	Peroxide value (mM O_2/kg ghee)				
	PG	U1	U2	U3	U4
0	0.00^{aA}	0.00^{aA}	0.00^{Aa}	0.00^{aA}	0.00^{aA}
15	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
30	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
45	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
60	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
75	4.27 ± 0.27^{bB}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
90	5.87 ± 0.53^{cB}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
105	7.47 ± 0.46^{dB}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
120	9.07 ± 0.53^{eC}	0.00^{aA}	0.00^{aA}	0.00^{aA}	3.60 ± 0.27^{bB}
135	10.13 ± 0.53^{eC}	0.00^{aA}	0.00^{aA}	3.2 ± 0.27^{bB}	4.80 ± 0.27^{cB}
150	11.73 ± 0.46^{fD}	0.00^{aA}	2.80 ± 0.27^{bB}	4.53 ± 0.27^{cBC}	5.60 ± 0.27^{dC}
165	12.80 ± 0.53^{fgE}	1.60 ± 0.27^{bA}	3.60 ± 0.27^{cB}	5.20 ± 0.27^{dC}	6.80 ± 0.27^{eD}
180	13.87 ± 0.53^{ghD}	2.40 ± 0.27^{cA}	4.40 ± 0.27^{dAB}	6.00 ± 0.27^{eBC}	8.00 ± 0.27^{fC}
195	14.40 ± 0.27^{hiE}	3.20 ± 0.27^{dA}	6.40 ± 0.27^{eB}	7.20 ± 0.27^{fC}	9.60 ± 0.27^{gD}
210	15.47 ± 0.53^{ijD}	4.00 ± 0.27^{eA}	7.60 ± 0.27^{fAB}	8.00 ± 0.27^{gBC}	11.20 ± 0.27^{hC}
225	16.53 ± 0.46^{jkD}	4.80 ± 0.27^{fA}	8.40 ± 0.46^{gAB}	9.20 ± 0.27^{hB}	12.80 ± 0.46^{iC}
240	17.60 ± 0.53^{klE}	6.00 ± 0.27^{gA}	9.60 ± 0.27^{hB}	10.40 ± 0.46^{iC}	14.40 ± 0.53^{jD}
255	18.73 ± 0.53^{lD}	7.60 ± 0.46^{hA}	11.2 ± 0.46^{iB}	12.00 ± 0.53^{jC}	16.40 ± 0.71^{kC}
270	20.40 ± 0.71^{mD}	9.60 ± 0.46^{iA}	12.40 ± 0.53^{jB}	14.00 ± 0.71^{kB}	18.40 ± 0.71^{lC}

Values are represented as Mean ± SD, n=3; $^{a, b \& A, B}$ – different superscript row-wise and Column-wise, respectively differ significantly ($P<0.05$)
PG: Pure Ghee; U1: PG spiked with TBHQ @ 0.02%; U2: PG spiked with TBHQ @ 0.01%; U3: PG spiked with TBHQ @ 0.005%; U4: PG spiked with TBHQ @ 0.0025%

3.1.4. Effect of storage period on the peroxide value of PG and BHA spiked PG samples at ambient temperature (30 ºC)

These results have been depicted in Table 5.0. It is evident from the data that peroxide value of PG sample as well as ghee sample containing lowest level of BHA i.e., V4 remained unaltered till 60 days of storage at ambient temperature. On the contrary, in case of ghee samples spiked with BHA @ 0.005, 0.01 and 0.02%, the increase in peroxide value was observed after 75, 90 and 105 days, respectively. It is also evident from the results that on account of storage there was increase in the peroxide value of all types of the samples but this increase was lowest and slowest in case of ghee samples containing BHA @ 0.02% and increase was corresponding to the added BHA in the ghee. The peroxide value of control PG significantly increased ($P<0.05$) from 0 on 0 days to 20.40.73±0.71 after 9 months (270 days).

Statistical analysis (Table 12.0) illustrated that the treatment i.e., BHA spiked ghee samples had significant effect on the peroxide value similarly, the storage days had also significant effect on peroxide value. The interaction effect between treatment and storage days on the development of peroxide value was significant at 95% level of confidence with *p*- value <0.001. The higher development of peroxide value in PG sample may be due to the more primary oxidation products formed in lack of antioxidant. Similar results were reported when ghee was treated with BHA and orange peel extract (Asa *et al*., 2015 and Rahila, 2016) [9].

Table 4: Effect of storage period on the peroxide value of PG and BHA spiked PG samples at ambient temperature (30 ºC)

Storage Days	Peroxide value (mM O_2/kg ghee)				
	PG	V1	V2	V3	V4
0	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
15	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
30	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
45	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
60	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}	0.00^{aA}
75	4.27 ± 0.27^{bB}	0.00^{aA}	0.00^{aA}	0.00^{aA}	4.00 ± 0.46^{bB}
90	5.87 ± 0.53^{cC}	0.00^{aA}	0.00^{aA}	3.60 ± 0.46^{bB}	5.33 ± 0.27^{cB}
105	7.47 ± 0.46^{dD}	0.00^{aA}	3.20 ± 0.46^{bB}	4.80 ± 0.27^{cBC}	6.93 ± 0.27^{dC}
120	9.07 ± 0.53^{eD}	2.00 ± 0.46^{bA}	4.00 ± 0.27^{bAB}	5.60 ± 0.27^{dBC}	8.00 ± 0.27^{eC}
135	10.13 ± 0.53^{eD}	2.80 ± 0.27^{bA}	4.80 ± 0.27^{cAB}	6.80 ± 0.27^{eBC}	9.20 ± 0.27^{fC}
150	11.73 ± 0.46^{fD}	3.60 ± 0.27^{cA}	6.00 ± 0.27^{dAB}	8.00 ± 0.27^{fB}	10.40 ± 0.00^{gC}
165	12.80 ± 0.53^{fgC}	4.40 ± 0.27^{dA}	7.20 ± 0.27^{eA}	9.20 ± 0.00^{gB}	11.47 ± 0.46^{hB}
180	13.87 ± 0.53^{ghD}	5.60 ± 0.27^{eA}	8.40 ± 0.00^{fAB}	10.40 ± 0.46^{hB}	12.27 ± 0.46^{iC}

195	14.40±0.27^{hiD}	6.80±0.00^{fA}	9.60±0.46^{gA}	11.60±0.46^{iB}	13.87±0.27^{jC}
210	15.47±0.53^{ijD}	8.00±0.46^{gA}	10.80±0.46^{hB}	12.80±0.27^{jBC}	14.67±0.27^{kC}
225	16.53±0.46^{jkD}	9.60±0.46^{hA}	12.00±0.27^{iAB}	14.00±0.27^{kBC}	15.47±0.27^{lC}
240	17.60±0.53^{klE}	11.20±0.27^{iA}	13.60±0.27^{jB}	15.60±0.27^{lC}	16.53±0.27^{mD}
255	18.73±0.53^{lD}	12.80±0.27^{jA}	15.20±0.27^{kAB}	16.80±0.27^{mBC}	17.60±0.27^{nC}
270	20.40±0.71^{mD}	14.40±0.27^{kA}	16.80±0.27^{lAB}	18.40±0.27^{nBC}	19.20±0.27^{nC}

Values are represented as Mean ± SD, n=3; $^{a, b \& A, B}$ – different superscript row-wise and column-wise, respectively differ significantly (P<0.05)
PG: Pure Ghee; V1: PG spiked with BHA @ 0.02%; V2: PG spiked with BHA @ 0.01%; V3: PG spiked with BHA @ 0.005%; V4: PG spiked with BHA @ 0.0025%

3.2. Effect of storage period on the TBA-value

TBA value test measures the secondary oxidation products of lipid oxidation. TBA value expressed as malondialdeyde (MDA) (mg %) in 0.1 g of fat. Therefore, the stored samples were evaluated for their status of oxidation by TBA- value at every 15 days interval up to 9 months.

3.2.1 Effect of storage period on the TBA-value of PG, PO and PO spiked PG samples at ambient temperature (30 ºC)

TBA value was determined and expressed as TBARS/0.1g of ghee. These results have been depicted in Table 5.0. It is evident from the data that PG as well as PO and PO spiked with PG samples showed an increase in the TBA value from the 0 days onwards. It is also evident from the data (Table 5,0) that PO had the TBA value (0.007±0.001) on zero day of storage. It was also observed that in spiked ghee samples, the TBA value significantly increased (P<0.05) as the storage period progressed and increase was corresponding to the level of added PO in the ghee. The TBA value of control PG significantly increased (P<0.05) from 0.019±0.001 on 0 days to 0.073±0.004 after 9 months (270 days), whereas, TBA value of PO was increased to 0.040±0.004 on 270 days of the storage. This increase was lower than those of PO spiked PG samples in the order Sa, Sb, Sc, Sd and Se (0.061±0.003, 0.064±0.002, 0.066±0.002, 0.068±0.001 and 0.070±0.003), respectively

The result in Table 5 indicated that the treatment i.e., palm oil spiked ghee samples had significant effect on the TBA value, similarly storage days had also significant effect on the TBA value. The interaction effect between treatment and storage days on the development of TBA was significant at 95% level of confidence with p- value <0.001. Comparatively high TBA value of ghee may be attributed to the fact that pure ghee did not contain any added synthetic antioxidant, whereas PO and PO spiked ghee samples had the prevention of oxidation due to the antioxidant present in PO. The SFA is of PO and PG is close and varied in the PO (48-50%), whereas in case of PG (65-66%). The PUFA of both PO and PG is also close and varied from (10-11%) and (4-5%), respectively (Montoya *et al.*, 2014; Pena-Serna and Restrepo-Betancur, 2020) [7, 8].

Table 5: Effect of storage period on the TBA-value of PG, PO and PO spiked PG samples at ambient temperature (30 ºC)

Storage Days	TBA-value (TBARS/0.1g ghee)						
	PG	PO	Sa	Sb	Sc	Sd	Se
0	0.019±0.001^{Ab}	0.007±0.001^{aA}	0.015±0.001^{aB}	0.016±0.001^{aBC}	0.017±0.001^{aCD}	0.018±0.001^{aD}	0.018±0.002^{aCD}
15	0.020±0.002^{bCD}	0.008±0.000^{bA}	0.016±0.000^{abB}	0.017±0.002^{abBC}	0.018±0.000^{abD}	0.019±0.002^{aD}	0.019±0.001^{abD}
30	0.021±0.001^{cE}	0.009±0.001^{abcA}	0.017±0.001^{bcB}	0.018±0.002^{bcC}	0.019±0.000^{bcCD}	0.020±0.000^{bDE}	0.020±0.001^{bDE}
45	0.022±0.001^{cdE}	0.010±0.001^{bcdA}	0.018±0.002^{cdB}	0.019±0.001^{cdBC}	0.020±0.002^{cdCD}	0.021±0.001^{bDE}	0.021±0.001^{bcDE}
60	0.023±0.001^{dD}	0.011±0.001^{cdA}	0.019±0.002^{deB}	0.020±0.001^{cdB}	0.021±0.001^{deC}	0.022±0.000^{bC}	0.023±0.001^{cdCD}
75	0.024±0.001^{dE}	0.012±0.002^{deA}	0.020±0.002^{efB}	0.021±0.000^{deBC}	0.022±0.001^{eCD}	0.023±0.002^{cDE}	0.024±0.001^{deDE}
90	0.026±0.003^{eD}	0.014±0.000^{efA}	0.022±0.001^{fB}	0.022±0.002^{eB}	0.024±0.001^{fC}	0.025±0.001^{dC}	0.025±0.001^{eC}
105	0.028±0.002^{fE}	0.016±0.002^{fgA}	0.023±0.001^{gB}	0.025±0.002^{fBC}	0.026±0.001^{gCD}	0.027±0.001^{deCD}	0.027±0.002^{fD}
120	0.031±0.002^{gF}	0.018±0.001^{gA}	0.025±0.001^{hB}	0.026±0.000^{fBC}	0.027±0.000^{gCD}	0.028±0.001^{eDE}	0.029±0.001^{fgE}
135	0.033±0.002^{gE}	0.020±0.003^{hA}	0.027±0.002^{iB}	0.028±0.001^{gB}	0.029±0.001^{hC}	0.030±0.001^{fCD}	0.031±0.000^{gD}
150	0.035±0.001^{hF}	0.022±0.002^{iA}	0.029±0.001^{jB}	0.030±0.001^{hBC}	0.031±0.003^{icCD}	0.032±0.001^{gD}	0.033±0.002^{hE}
165	0.037±0.001^{iE}	0.024±0.002^{ijA}	0.031±0.002^{kB}	0.032±0.002^{hB}	0.033±0.001^{jC}	0.034±0.001^{hCD}	0.035±0.003^{hD}
180	0.039±0.001^{jF}	0.026±0.001^{jkA}	0.033±0.001^{lB}	0.034±0.001^{iBC}	0.035±0.001^{kCD}	0.036±0.001^{iDE}	0.037±0.002^{iE}
195	0.042±0.002^{kD}	0.028±0.003^{klA}	0.035±0.002^{mB}	0.037±0.001^{jBC}	0.039±0.001^{lC}	0.039±0.001^{jC}	0.040±0.003^{jC}
210	0.046±0.003^{lE}	0.030±0.002^{lA}	0.038±0.002^{nB}	0.040±0.002^{kC}	0.042±0.001^{mD}	0.043±0.001^{kD}	0.044±0.001^{kD}
225	0.052±0.002^{mF}	0.032±0.003^{mA}	0.041±0.001^{oB}	0.044±0.002^{lC}	0.046±0.001^{nCD}	0.048±0.002^{lDE}	0.050±0.002^{lE}
240	0.058±0.002^{nF}	0.034±0.001^{nA}	0.045±0.001^{pB}	0.049±0.001^{mC}	0.052±0.003^{oD}	0.054±0.001^{mE}	0.056±0.001^{mE}
255	0.065±0.003^{oF}	0.037±0.002^{oA}	0.052±0.002^{qB}	0.056±0.002^{nC}	0.058±0.002^{pCD}	0.060±0.001^{nDE}	0.062±0.002^{nE}
270	0.073±0.004^{pF}	0.040±0.004^{pA}	0.061±0.003^{rB}	0.064±0.002^{oBC}	0.066±0.002^{qCD}	0.068±0.001^{oDE}	0.070±0.003^{oE}

Values are represented as Mean ± SD, n=3; $^{a, b \& A, B}$ – different superscript row-wise and column-wise, respectively differ significantly (P<0.05)
PG: Pure Ghee; PO: Palm Oil; Sa: PG spiked with PO @ 10%; Sb: PG spiked with PO @ 7.5%; Sc: PG spiked with PO @ 5%; Sd: PG spiked with PO @ 2.5%; Se: PG spiked with PO @ 1%;

3.2.2 Effect of storage period on the TBA-value of PG, SFO and SFO spiked PG samples at ambient temperature (30 ºC)

It is evident from the data (Table 6.0) that PG as well as SFO and SFO spiked PG samples showed an increase in the TBA value on account of storage. It is also evident from the data (Table 6.0) that sunflower oil had the TBA value (0099±0.001) on 0 day of storage. It was also observed that in both SFO and spiked ghee samples, the TBA value significantly increased (P<0.05) as the storage period progressed and increase was corresponding to the level of added SFO in the ghee. The TBA value of control PG significantly increased (P<0.05) from 0.019±0.001 on 0 days to 0.073±0.004 after 9 months (270 days), whereas, TBA

value of SFO was increased to 0.281±0.006 on 270 days of the storage vis'-a-vis' 0.099 ±0.001 on 0 day. This increase was higher than those of SFO spiked PG samples in the order Ta>Tb>Tc>Td>Te (0.094±0.001, 0.088±0.001, 0.084±0.001, 0.082±0.001, 0.081±0.001), respectively.

The result in Table 10.0 indicated that the treatment i.e. SFO spiked ghee samples had significant effect on the peroxide value, similarly storage days had also significant effect on the peroxide value. The interaction effect between treatment and storage days on the development of TBA was significant at 95% level of confidence with *p*- value <0.001. The higher development of TBA value in SFO and SFO spiked ghee samples (Sa, Sb, Sc, Sd and Se) than PG. The reason behind that the approx. 65-67% i., e higher percentage of poly unsaturated fatty acid available in SFO.

Gazwi (2017) [4] reported that the TBA value of sunflower oil increased significantly from (1.144-12.65) mg malondialdehyde/kg of oil from 1-20 days of storage at 65 ºC.

Table 6: Effect of storage period on the TBA value of PG, SFO and SFO spiked PG samples at ambient temperature (30 ºC)

Storage Days	TBA-value (TBARS/0.1g ghee)						
	PG	SFO	Ta	Tb	Tc	Td	Te
0	0.019±0.001^{Aa}	0.099±0.001^{aD}	0.022±0.001^{aC}	0.021±0.000^{aBC}	0.020±0.001a^{ABC}	0.019±0.000^{aABC}	0.019±0.001$^{\mathbf{aAB}}$
15	0.020±0.002^{bA}	0.104±0.001^{bD}	0.023±0.001^{bC}	0.022±0.001^{bBC}	0.021±0.001^{bABC}	0.020±0.002^{bAB}	0.020±0.00^{bAB}
30	0.021±0.001^{cA}	0.115±0.002^{cE}	0.026±0.001^{cD}	0.025±0.001^{cCD}	0.024±0.002^{cBC}	0.023±0.001^{cB}	0.022±0.001^{cAB}
45	0.022±0.001^{cdA}	0.123±0.001^{dE}	0.028±0.001^{cdD}	0.027±0.000^{cdCD}	0.025±0.002^{cdBC}	0.024±0.001^{cdAB}	0.023±0.002^{cA}
60	0.023±0.001^{dA}	0.127±0.001^{dD}	0.030±0.001^{deC}	0.029±0.002^{deC}	0.028±0.001^{dB}	0.025±0.001^{dAB}	0.024±0.003^{cdA}
75	0.024±0.001^{dA}	0.133±0.001^{eE}	0.032±0.001^{eD}	0.031±0.001^{eD}	0.029±0.001^{eC}	0.027±0.001^{eB}	0.026±0.001^{dA}
90	0.026±0.003^{eA}	0.145±0.001^{fF}	0.035±0.002^{fE}	0.034±0.001^{fDE}	0.032±0.001^{fCD}	0.031±0.000^{fBC}	0.029±0.001^{efAB}
105	0.028±0.002^{fA}	0.154±0.001^{gF}	0.037±0.001^{gE}	0.036±0.002^{fgDE}	0.035±0.001^{fgCD}	0.033±0.001^{gBC}	0.032±0.002^{gAB}
120	0.031±0.002^{gA}	0.163±0.001^{hE}	0.040±0.002^{hD}	0.038±0.001^{gC}	0.037±0.001^{gBC}	0.035±0.002^{gAB}	0.034±0.002^{gAB}
135	0.033±0.002^{gA}	0.175±0.005^{iE}	0.043±0.001^{hD}	0.041±0.001^{hCD}	0.039±0.001^{hBCD}	0.036±0.001^{hABC}	0.035±0.001^{hAB}
150	0.035±0.001^{hA}	0.184±0.002^{jE}	0.046±0.001^{iD}	0.043±0.003^{hC}	0.041±0.002^{iBC}	0.040±0.001^{hAB}	0.038±0.001^{iAB}
165	0.037±0.001^{iA}	0.199±0.002^{kE}	0.050±0.002^{jD}	0.045±0.001^{iC}	0.044±0.001^{jBC}	0.042±0.003^{iAB}	0.040±0.003^{iA}
180	0.039±0.001^{jAB}	0.202±0.001^{kF}	0.055±0.001^{kE}	0.051±0.002^{jD}	0.047±0.001^{kCD}	0.045±0.001^{jBC}	0.041±0.003^{jA}
195	0.042±0.002^{kA}	0.209±0.002^{lE}	0.060±0.002^{lD}	0.053±0.001^{kC}	0.051±0.001^{lC}	0.049±0.001^{kB}	0.048±0.001^{kAB}
210	0.046±0.003^{lA}	0.222±0.002^{mF}	0.066±0.001^{mE}	0.061±0.001^{lD}	0.059±0.001^{mD}	0.057±0.001^{lC}	0.054±0.001^{lB}
225	0.052±0.002^{mA}	0.239±0.003^{nF}	0.072±0.001^{nE}	0.067±0.001^{mD}	0.065±0.001^{nCD}	0.064±0.001^{mBC}	0.062±0.001^{mB}
240	0.058±0.002^{nA}	0.257±0.004^{oE}	0.080±0.002^{oD}	0.075±0.001^{nC}	0.073±0.001^{oBC}	0.070±0.001^{nAB}	0.068±0.001^{nA}
255	0.065±0.003^{oA}	0.272±0.004^{pE}	0.087±0.002^{pD}	0.082±0.002^{oC}	0.079±0.002^{pBC}	0.077±0.002^{oAB}	0.076±0.001^{oA}
270	0.073±0.004^{pA}	0.281±0.006^{qE}	0.094±0.001^{qD}	0.088±0.001^{pC}	0.084±0.001^{qBC}	0.082±0.001^{pAB}	0.081±0.001^{pAB}

Values are represented as Mean ± SD, n=3; $^{a, b \& A, B}$ – different superscript row-wise and column-wise, respectively differ significantly (P<0.05)
PG: Pure Ghee; SFO: Sunflower Oil; Ta: PG spiked with SFO @ 10%; Tb: PG spiked with SFO @ 7.5%; Tc: PG spiked with SFO @ 5%; Td: PG spiked with SFO @ 2.5%; Te: PG spiked with SFO @ 1%

3.2.3 Effect of storage period on the TBA-value of PG and TBHQ spiked PG samples at ambient temperature (30 ºC)

It is evident from the data (Table 7) that PG as well as TBHQ spiked ghee samples showed an increase in the TBA value from the 0 days onwards. It was observed that in TBHQ spiked ghee samples, the TBA value significantly increased (P<0.05) as the storage period progressed and increase was corresponding to the added TBHQ in the ghee. The TBA value of control PG significantly increased (P<0.05) from 0.019±0.001 on 0 days to 0.073±0.004 after 9 months (270 days). On the contrary, in case of TBHQ spiked ghee samples, this increase was lower than the ghee containing no added TBHQ. Even in the lowest added concentration of TBHQ i.e. 0.0025%, the TBA value measured after 270 days of storage was 0.041±0.001, which was just the half of the value reported in PG samples without any TBHQ. This clearly indicated that the addition of TBHQ helped in controlling the rate of oxidation of ghee during storage.

The result of ANOVA (Table 11.0) illustrated that the treatment i.e., TBHQ spiked ghee samples had significant effect on the TBA value. Similarly, the storage days had also significant effect on TBA value. The interaction effect between treatment and storage days on the development of TBA was significant at 95% level of confidence with *p*- value <0.001. The PG sample highest increase in TBA value may be due to the more secondary oxidation products formed during storage.

Table 7: Effect of storage period on the TBA-value of PG and TBHQ spiked PG samples at ambient temperature (30 °C)

Storage Days	TBA-value (TBARS/0.1g ghee)				
	PG Pure	Ua	Ub	Uc	Ud
0	0.019±0.001^{aC}	0.006±0.001^{aA}	0.007±0.001^{aA}	0.009±0.001^{aB}	0.014±0.001^{aC}
15	0.020±0.002^{bD}	0.007±0.001^{aA}	0.008±0.000^{bcA}	0.012±0.002^{abB}	0.016±0.001^{aC}
30	0.021±0.001^{cD}	0.007±0.002^{aA}	0.009±0.001^{cA}	0.013±0.001^{bB}	0.017±0.001^{bC}
45	0.022±0.001^{cdC}	0.009±0.002^{abA}	0.010±0.001^{dA}	0.015±0.001^{cB}	0.019±0.001^{bcB}
60	0.023±0.001^{dD}	0.011±0.001^{bcA}	0.012±0.001^{eB}	0.016±0.001^{cdC}	0.020±0.001^{cC}
75	0.024±0.001^{dD}	0.011±0.001^{bcA}	0.014±0.001^{fB}	0.018±0.001^{dC}	0.021±0.002^{dC}
90	0.026±0.003^{eD}	0.013±0.001^{cdA}	0.017±0.001^{fgB}	0.020±0.001^{deBC}	0.022±0.001^{dC}
105	0.028±0.002^{fE}	0.014±0.001^{deA}	0.019±0.001^{gB}	0.021±0.002^{eC}	0.025±0.001^{eD}
120	0.031±0.002^{gD}	0.016±0.003^{efA}	0.021±0.001^{hB}	0.025±0.001^{fC}	0.028±0.003^{fC}
135	0.033±0.002^{gD}	0.018±0.003^{fgA}	0.022±0.001^{iB}	0.027±0.003^{gC}	0.030±0.003^{gC}
150	0.035±0.001^{hD}	0.019±0.005^{ghA}	0.024±0.002^{ijB}	0.029±0.003^{ghBC}	0.031±0.003^{ghC}
165	0.037±0.001^{iD}	0.022±0.003^{hiA}	0.026±0.002^{jkB}	0.031±0.003^{hiC}	0.032±0.002^{hiC}
180	0.039±0.001^{jE}	0.023±0.002^{hijA}	0.027±0.002^{klB}	0.031±0.001^{hiC}	0.033±0.001^{hiD}
195	0.042±0.002^{kE}	0.025±0.002^{ijA}	0.028±0.001^{lmB}	0.032±0.001^{ijC}	0.034±0.002^{ijD}
210	0.046±0.003^{lE}	0.025±0.002^{jkA}	0.028±0.002^{mB}	0.032±0.001^{ijC}	0.035±0.002^{ijD}
225	0.052±0.002^{mE}	0.026±0.001^{jkA}	0.029±0.001^{mnB}	0.033±0.003^{ijC}	0.036±0.001^{jkD}
240	0.058±0.002^{nE}	0.027±0.001^{klA}	0.030±0.001^{nB}	0.034±0.003^{jC}	0.037±0.001^{klD}
255	0.065±0.003^{oD}	0.029±0.001^{lA}	0.032±0.001^{nA}	0.035±0.001^{jB}	0.038±0.00l^{lC}
270	0.073±0.004^{Pe}	0.030±0.002^{lA}	0.034±0.001^{oB}	0.036±0.002^{kC}	0.041±0.001^{mD}

Values are represented as Mean ± SD, n=3; $^{a, b \& A, B}$ – different superscript row-wise and column-wise, respectively differ significantly (P<0.05)
PG: Pure Ghee; Ua: PG spiked withTBHQ @ 0.02%; Ub: PG spiked with TBHQ @ 0.01%; Uc: PG spiked with TBHQ @ 0.005%; Ud: PG spiked with TBHQ @ 0.0025%

3.2.4 Effect of storage period on the TBA-value of PG and BHA spiked PG samples at ambient temperature (30 ºC)

These results have been depicted in Table 8. It is evident from the data that PG as well as TBHQ spiked ghee samples showed an increase in the TBA value from the 0 days onwards. It was observed that in TBHQ spiked ghee samples, the TBA value significantly increased (P<0.05) as the storage period progressed and increase was corresponding to the added TBHQ in the ghee. The TBA value of control PG significantly increased (P<0.05) from 0.019±0.001 on 0 days to 0.073±0.004 after 9 months (270 days). This increase was lower than those of TBHQ spiked PG samples in the order Va, Vb, Vc and Vd (0.052±0.001, 0.056±0.003, 0.060±0.002, 0.070±0.001), respectively.

The result in Table 12.0 illustrated that the treatment i.e., BHA spiked ghee samples had significant effect on the TBA value similarly, the storage days had also significant effect on TBA value. The interaction effect between treatment and storage days on the development of TBA was significant at 95% level of confidence with p- value <0.001. The PG sample highest increase in TBA value may be due to the more secondary oxidation products formed during storage. Similar reason drawn when they added BHA and natural antioxidant like orange peel extract in ghee Asa *et al*., (2015), BHA incorporated into ghee Rahila (2016) [9].

Rahila (2016) [9] also reported that the peroxide value of cow ghee added with BHA (synthetic antioxidant) during storage increased for 1-210 days of storage at 37±1 °C and 1-60 days of storage at 60±1 °C from (0.00-19.69) and (0.00-23.02) mM O_2/kg of ghee, respectively.

Table 8: Effect of storage period on the TBA-value of PG and BHA spiked PG samples at ambient temperature (30 °C)

Storage Days	TBA-value (TBARS/0.1g ghee)				
	PG Pure	Va	Vb	Vc	Vd
0	0.019±0.001^{aB}	0.010±0.001^{aA}	0.014±0.001^{aB}	0.015±0.001^{aB}	0.016±0.001^{aB}
15	0.020±0.002^{bC}	0.011±0.001^{aA}	0.015±0.002^{bB}	0.017±0.001^{bBC}	0.017±0.001^{bBC}
30	0.021±0.001^{cC}	0.012±0.001^{bA}	0.016±0.001^{cB}	0.018±0.001^{bB}	0.019±0.001^{cBC}
45	0.022±0.001^{cdC}	0.015±0.002^{cA}	0.018±0.002^{cB}	0.020±0.001^{cB}	0.021±0.002^{cdBC}
60	0.023±0.001^{dC}	0.016±0.001^{cdA}	0.020±0.002^{dB}	0.021±0.001^{cB}	0.021±0.001^{deB}
75	0.024±0.001^{dC}	0.017±0.001^{cdA}	0.021±0.002^{deB}	0.022±0.001^{dB}	0.023±0.001^{eB}
90	0.026±0.003^{eD}	0.018±0.000^{dA}	0.023±0.003^{efB}	0.024±0.001^{eBC}	0.025±0.001^{fC}
105	0.028±0.002^{fD}	0.019±0.001^{eA}	0.024±0.002^{fB}	0.027±0.001^{fC}	0.028±0.001^{gC}
120	0.031±0.002^{gD}	0.020±0.001^{eA}	0.027±0.002^{gB}	0.029±0.001^{gC}	0.030±0.002^{hC}
135	0.033±0.002^{gD}	0.022±0.001^{fA}	0.030±0.001^{hB}	0.032±0.001^{hBC}	0.032±0.001^{iCD}
150	0.035±0.001^{hE}	0.023±0.002^{fA}	0.031±0.001^{hiB}	0.033±0.002^{hC}	0.034±0.001^{jD}
165	0.037±0.001^{iD}	0.025±0.001^{gA}	0.033±0.001^{iB}	0.034±0.001^{iB}	0.036±0.002^{kC}
180	0.039±0.001^{jD}	0.028±0.001^{hA}	0.036±0.001^{jB}	0.037±0.001^{jB}	0.038±0.001^{lC}
195	0.042±0.002^{kE}	0.031±0.001^{iA}	0.037±0.003^{jB}	0.039±0.001^{kC}	0.040±0.001^{mD}
210	0.046±0.003^{lD}	0.032±0.002^{jA}	0.043±0.002^{kB}	0.045±0.001^{lB}	0.045±0.001^{nC}
225	0.052±0.002^{mE}	0.038±0.001^{kA}	0.046±0.003^{lB}	0.049±0.002^{mC}	0.050±0.002^{oD}
240	0.058±0.002^{nE}	0.044±0.001^{lA}	0.048±0.001^{mB}	0.053±0.001^{nC}	0.055±0.001^{pD}
255	0.065±0.003^{oE}	0.047±0.001^{mA}	0.050±0.002^{nB}	0.055±0.001^{oC}	0.063±0.001^{qD}
270	0.073±0.004^{pE}	0.052±0.001^{nA}	0.056±0.003^{oB}	0.060±0.002^{pc}	0.070±0.001^{rD}

Values are represented as Mean ± SD, n=3; $^{a, b \& A, B}$ – different superscript row-wise and column-wise, respectively differ significantly (P<0.05)
PG: Pure Ghee; Va: PG spiked with BHA @ 0.02%; Vb: PG spiked with BHA @ 0.01%; Vc: PG spiked with BHA @ 0.005%; Vd: PG spiked with BHA @ 0.0025%

Table 9: Two-way ANNOVA table of pure ghee, Palm oil and ghee spiked with PO different levels

Source	Peroxide Value				TBA Value				Disc color change time			
	Df	Mean Square	F	Sig.	Df	Mean Square	F	Sig.	Df	Mean Square	F	Sig.
Treatment(T)	6	3384.881	6299.289	.000	.011	6	.002	.000	6	958195.042	42324.789	.000
Days(D)	18	1295.597	2411.115	.000	.077	18	.004	.000	18	4957.880	218.996	.000
TxD	108	3.739	6.959	.000	108	2.535E-005	21.075	.000	108	1427.974	63.076	.000
Error	266	.537			266	1.203E-006			266	22.639		
Total	399				399				399			

Table 10: Two-way ANNOVA table of pure ghee, Sunflower oil and ghee spiked with SFO different levels

Source	Peroxide Value				TBA Value				Disc color change time			
	Df	Mean Square	F	Sig.	Df	Mean Square	F	Sig.	Df	Mean Square	F	Sig.
Treatment(T)	6	2360.027	5523.837	.000	6	.151	63926.438	.000	6	826476.854	152474.518	.000
Days(D)	18	1163.189	2722.539	.000	18	.014	5747.619	.000	18	3420.606	631.059	.000
TxD	108	2.294	5.370	.000	108	.001	253.743	.000	108	1328.753	245.138	.000
Error	266	.427			266	2.361E-006			266	5.420		
Total	399				399				399			

Table 11: Two-way ANNOVA table of pure ghee and ghee spiked with TBHQ at different levels

Source	Peroxide Value				TBA Value				Disc color change time			
	Df	Mean Square	F	Sig.	Df	Mean Square	F	Sig.	Df	Mean Square	F	Sig.
Treatment(T)	4	431.139	2232.459	.000	4	.004	2507.606	.000	4	1214573.421	176970.054	.000
Days(D)	18	295.079	1527.934	.000	18	.002	1012.735	.000	18	1797.845	261.956	.000
TxD	72	10.380	53.750	.000	72	8.931E-005	58.115	.000	72	1776.332	258.821	.000
Error	190	.193			190	1.537E-006			190	6.863		
Total	285				285				285			

Table 12: Two-way ANNOVA table of pure ghee and ghee spiked with BHA at different levels

Source	Peroxide Value				TBA Value				Disc color change time			
	Df	Mean Square	F	Sig.	Df	Mean Square	F	Sig.	Df	Mean Square	F	Sig.
Treatment(T)	4	173.994	679.662	.000	4	.002	1555.047	.000	4	1137573.833	155719.761	.000
Days(D)	18	452.487	1767.529	.000	18	.003	3290.711	.000	18	1797.845	261.956	.000
TxD	72	4.392	17.158	.000	72	2.826E-005	27.772	.000	72	1649.746	225.830	.000
Error	190	.256			190	1.018E-006			190	7.305		
Total	285				285				285			

4. Conclusion

During the storage period of 9 months, peroxide value and TBA value of both the vegetable oils used in the study increased rapidly because of more unsaturated fatty acids. Peroxide value of all the samples increased during the storage period and this increase was observed in the following order: PO >SFO> PO spiked PG> SFO spiked PG>PG>BHA spiked PG> TBHQ spiked PG. TBA value of all the samples increased during the storage period and this increase was observed in the following order: SFO> SFO spiked PG> PG> PO spiked PG> PO>BHA spiked PG> TBHQ spiked PG.

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