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Lycopene Extraction and Application as a Natural Antioxidant for the Preservation of Ghee in Surplus Tomatos (*Solanum Lycopersicum L.)*

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Abstract:- The current study aims to regulate overabundance tomatoes by exploring the possibility of tomatoes' inherent antioxidants to safeguard lipid or fat-containing dietary items. Ghee loses quality as it gets heated because peroxide and free fatty acids are formed, among other things. Consequently, it is imperative to investigate methods and strategies to halt or reduce ghee's degradation. The purpose of the current investigation was to ascertain the solvent extraction yield of lycopene produced from tomatoes and the efficacy of lycopene in extending the shelf life of ghee relative to commonly used synthetic antioxidants. The experiment was conducted with six different concentrations of lycopene (0.01% w/w, 0.02% w/w, 0.05% w/w, 0.1% w/w, 0.25% w/w and 0.5% w/w) with ghee. The samples were cooled for two hours between each of the first three heat treatments (180 ± 50 C for 15 minutes). Percent inhibition values for radical scavenging activity (DPPH) gradually decreased as the number of heat treatments increased. The lowest decrease was observed with BHA (0.02%), and the maximum decrease was observed with control (unblended). However, up to 49 days, the performance of lycopene (0.02%) was also comparable to that of BHA. All heat treatments did not, however, cause BHA (0.02%) or lycopene (0.02%) to rise in peroxide levels in a same way. As the 49-day period went on, a similar pattern was also seen in the free fatty acid percentage. Being a naturally occurring antioxidant, lycopene (0.02%) may therefore be advised for the preservation of ghee following heat treatments.

Keywords:- *BHA; DPPH; Peroxide Value; Free Fatty Acid; Lycopene.*

I. INTRODUCTION

Tomato is botanically known as Solanum lycopersicum L. and it belongs to the family of Solanaceae. While tomatoes are botanically berry-type fruits, they are considered as a culinary vegetable, being ingredients of savory meals. Tomato contains 95% water, 4% carbohydrates and less than 1% of fat and protein. In 2022-23, India's tomato production was 206.2 lakh tonnes, a 0.35% decrease from 2021-22. However, the cultivation area for tomatoes in India increased slightly in 2023, reaching 864 thousand hectares. Andhra Pradesh, Orissa, Karnataka, Madhya Pradesh, West Bengal, Bihar, and Maharashtra are the states that produce the majority of tomatoes. With an average productivity of 31.0 t/ha, Madhya Pradesh is the state that produces the most tomatoes, but Karnataka has the greatest average productivity (31.7 t/ha), mostly because of the adoption of high-yielding hybrids and conducive climatic conditions. There is a considerable output and surplus as a result of overstock and waste during the season, which must be reduced for the benefit of the national economy. There are several strategies to minimize it. Ghee's shelf life can be increased by extracting and using lycopene, a naturally occurring antioxidant. The longest carbon chain of any carotenoid is found in lycopene, a highly unsaturated hydrocarbon with 11 conjugated and 2 non-conjugated double bonds. The main cause of lycopene degradation during food preparation is cis-trans-isomerization, which occurs when lycopene is a polyene. Typically, ghee is kept at room temperature (25–30˚C) and packed full in a rust-proof container. Ghee experiences an increase in rancidity over time due to both oxidative and hydrolytic processes. The extent of rancidity is influenced by the storage conditions, such as the level of exposure to air and light, the presence of metallic contaminants like iron and copper, and the availability or absence of pro-oxidants and antioxidants. Properly clarified ghee typically exhibits minimal hydrolytic rancidity, with oxidative rancidity being the primary factor affecting the flavor degradation of ghee. To combat oxidative rancidity and extend the shelf life of ghee, the dairy industry is utilizing artificial antioxidants. According to the Food Safety and Standards Regulations (FSSR) of 2011, butylated hydroxyanisole (BHA) can be added to ghee as an artificial antioxidant, with a maximum allowable concentration of 0.02%. However, because consumers prefer organically produced antioxidants because they are seen to be safe, natural, and healthful, there is a significant interest in using these antioxidants globally.

➢ *Justification*

The growing knowledge of the advantageous health effects of natural substances is driving the demand for natural antioxidants. The use of natural ingredients in culinary applications is trending globally. Human health is affected by allergies and cancer when artificial antioxidants are used in food. In order to meet consumers demand for natural additives, the present study was formulated to utilize the potential of lycopene as a natural antioxidant with the following objectives:

- To find out the extraction yield of solvent extracted lycopene from tomato, and
- To find out the efficacy of lycopene for enhancing the shelf life of ghee compared to a commonly used synthetic antioxidant.

II. MATERIAL AND METHODS

The red ripe tomatoes were procured from local Tech Market, IIT, Kharagpur and the chemicals used in the project were procured from Kolkata chemical supplier (hexane, acetone, ethanol, Butyl hydroxyanisole (BHA), ammonium molybdate and sodium phosphate).

A. Solvent Extraction of Lycopene Pigment

The tomato sample was rinsed with tap water, then wiped dry and chopped into small pieces before being blended into a paste in the laboratory. A quantity of 100g of the tomato paste was placed in a 250 ml beaker, then heated, and approximately 30 ml of warm hexane (at 40°C) was added. The mixture was stirred thoroughly on a magnetic stirrer for about 3-4 minutes, and then the aqueous layer was decanted. An additional 30 ml of warm solvent was added, stirred, and the layer was decanted again. This process was repeated around 4-5 times. The decanted samples containing lycopene were then placed in a Socsplus apparatus to evaporate the solvents at 80°C for up to 2 hr and 30 mins. Then obtained lycopene was dissolved in 10 ml hexane for the further analysis (Fish W *et al*., 2003). Identification of chemical structure was done using visible spectrophotometer (Malviya, 2014).

B. Equipment Used

- Socsplus- SCS AS DLS: This was used to evaporate the solvent traces from the solutions.
- Spectrophotometer (Epoch 2, BioTek, USA): The 96-well microplate spectrophotometer was used for UV-visible absorption spectrum of lycopene pigment.

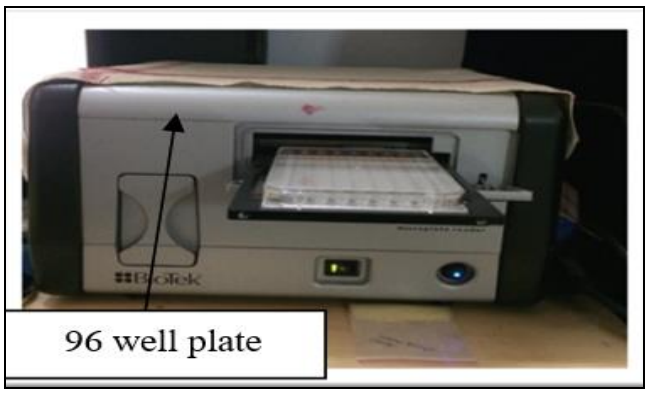

Fig 1: Spectrophotometer for UV- visible spectral analysis


Fig 2: Socsplus Apparatus used for Evaporation of Solvent

C. Determination of Total Lycopene Content

The lycopene content of the extracted pigment was calculated by equation 3.1 at peak absorbance wavelength of 472 nm.

Where molar absorbance coefficient of hexane in lycopene at 472

$$\text{Lycopene}\,(\%) = \frac{\text{Absorbance at 472 nm}}{3450 \times l \times \text{specimen weight(g)}} \times \text{Dilution factor} \times 100$$

nm is 3450 $M^{-1}cm^{-1}$ and *l* is the path length which is 0.398 cm.

D. Total Antioxidant Capacity

This assay is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH (Prieto *et al.*, 1999). A 0.1 ml aliquot of the sample solution, which included 100–500 µg of dried extract in the appropriate solvent, was mixed with 1 ml of the reagent solution (0.6 M sulfuric acid, 28 milli-molar sodium phosphate, and 4 milli-molar ammonium molybdate) in an Eppendorf tube. After being sealed, the tubes were incubated for 90 minutes at 95 °C in a thermal block. The absorbance was measured at 695 nm against a blank after the samples had cooled to room temperature. A standard blank solution was incubated in the same conditions as the other samples, using 1 ml of reagent solution and the appropriate amount of the same solvent used for the sample. The antioxidant activity of extracts was expressed as equivalents of α-tocopherol using

extinction coefficient of 4 × 10^3 M^{-1} cm^{-1} and was calculated by following equation 2.2.

$$C = \frac{A}{\varepsilon l}$$

Where, C correspond to total antioxidant capacity in terms of α-tocopherol, A is the absorbance at 695 nm, *l* is the depth of sample in 96 well plate (0.398 cm) and **ε** is the molar extinction coefficient of α-tocopherol.

E. Sensory Evaluation

The sensory evaluation of ghee samples blended with lycopene extracted from tomato in different concentrations of 0.01% (w/w), 0.02% (w/w), 0.05% (w/w), 0.10% (w/w), 0.25% (w/w), 0.5% (w/w) and control was done by 9-point hedonic scale method as described by Silva et al., (2013).

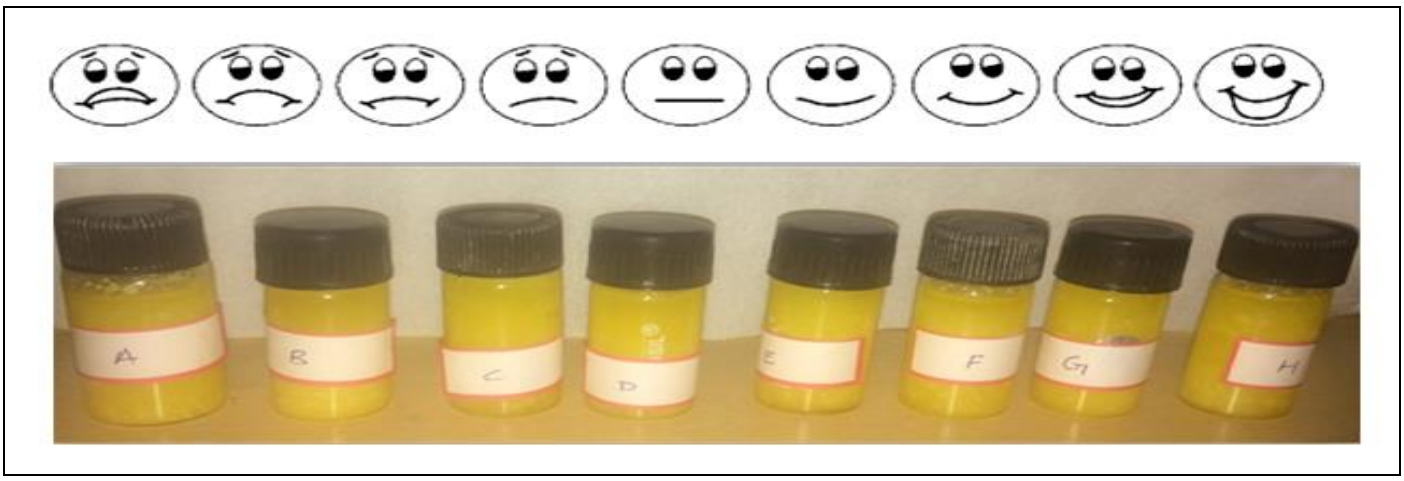

Fig 3: Sensory Evaluation of Different Concentration of Ghee

Table 1: Representation of 9-Point Hedonic Scale Ranging from Dislike Extremely to Like Extremely

1- Dislike Extremely	**2- Dislike Very Much**	**3 - Dislike Moderately**
4- Dislike Slightly	5- Neither Like nor Dislike	6- Like Slightly
7- Like Moderately	8- Like Very Much	7- Like Extremely

Using a 9-point hedonic scale with values associated to the hedonic term ranging from "disliked extremely" to "liked extremely" and a central hedonic term of "neither liked nor disliked" with a value of 1, 9, and 5 respectively, this scale was referred to as "1 to 9". The core purpose of sensory analysis is to measure sensory characteristics as they would be perceived by a human, such as color, odour, flavor, and taste. This scale was referred to as "1 to 9". The information obtained from the sensory analysis is unique and differs clearly from other sources of information in which chemicals or instruments are used to characterize food.

F. Effect of Thermal Treatment on Physicochemical Attributes of Ghee

While undergoing thermal therapy Ghee samples, namely control ghee, ghee blended with lycopene, and ghee blended with BHA, weighing 600g each, were placed in separate jars and cooked for 15 minutes at 180 ± 50C on a heating mantle. A thermometer was used to monitored the temperature of ghee. The entire heating cycle was performed three times (the first, second, and third treatments) using a comparable heat treatment. After the initial treatment, the material was allowed to cool to ambient temperature before undergoing a second treatment. As a result, each of the two treatments was separated by two hours.

Fig 4: Blended Ghee after Three Thermal Treatment

G. Determination of Free Radical Scavenging Activity

The stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to bleach a purple-colored ethyl ethanolic solution in order to quantify the hydrogen-donating or radical scavenging capabilities of lycopene. Blois (1958) technique was used to assess the radical-scavenging activity of lycopene and butylated hydroxyanisole (BHA) in ghee. The diluted solutions were prepared in ethanol. The decrease in absorbance at 517 nm was determined using a spectrophotometer for all samples. Ethanol was used to zero the spectrophotometer. It was determined by the following equation 3.3.

$$\% \text{ Inhibition} = (A_{blank} - A_{sample})/A_{blank} \times 100 \quad \text{.....(3.3)}$$

Where, A_{blank} corresponds to the absorbance of blank at t= 0 min and A_{sample} is absorbance of the samples after incubation (30 min).

H. Determination of Peroxide Value

This illustrates how much oxidation the oil has undergone. The peroxide levels of the ghee samples were ascertained using the FSSAI Rule, 2015 procedure. Weighing a 5 g sample into a 250 ml conical flask with a cap was all that was required. Add 30 ml of the acetic acid-chloroform solvent mixture and swirl to dissolve it. Add 0.5 ml of saturated potassium iodide solution using a Mohr pipette. Add about thirty milliliters of water after standing in the dark for a minute while shaking occasionally. While shaking a 0.1 N sodium thiosulphate solution constantly, gradually titrate the released iodine until the yellow tint is nearly gone. Following the addition of approximately 0.5 ml of starch solution as an indicator, repeat the titration and vigorously shake the mixture to ensure the complete release of I2 from the chloroform layer until the blue color disappears. The determination of blanks was carried out. The peroxide value was calculated using equation 3.4 and was represented as the milliequivalent of peroxide oxygen per kg sample (meq/kg).

$$\text{Peroxide value} = (T \times N \times 1000)/m \quad \text{.....} \quad (3.4)$$

Where, T is the sample weight in kilograms; N is the sodium thio-sulphate solution's normalcy; and m is the sample weight in milligrams.

I. Free Fatty Acid Content Determination

The amount of potassium hydroxide milligrams needed to neutralize the free fatty acids in one gram of fat is known as the acid value. The FSSAI Rule (2015) method was used to determine the FFA levels of ghee samples. Ten grams of the ghee sample should be weighed in a 250 ml conical flask. While the temperature is still over 70 °C, bring 50 ml of ethanol to a boil in a different flask. Then, use 0.5 ml of 0.1 N NaOH to neutralize the ethanol. Add the neutralized alcohol to a flask containing a sample of ghee and mix the contents. Titrate the mixture with 0.1 N NaOH while it's still hot after bringing it to a boil, giving it a good shake throughout. Free fatty acid may be calculated using equation 3.5. The titration reaches its endpoint when the addition of a single drop results in a minor but noticeable color shift that lasts for at least 15 seconds.

$$\text{Free Fatty Acid (as oleic acid)} = (T \times 2.82)/m \quad \text{.....(3.5)}$$

Where, T = Titre volume of 0.1 N alkali required for titration in ml, m = mass of sample in g.

J. Colour Measurement

The color of food samples and the color change that takes place during processing are measured using a chromata meter (model no. CR-400 was utilized in this experiment). The measured color is represented by the color notation L*, a*, and b*. L*, which is equal to zero for black and 100 for white, is the color's lightness. A* represents the degree of redness (0 to 60) or greenness (0 to -60), while b* represents the amount of yellowness (0 to 60) or blueness (0 to -60). This is a 3-D color display system.

K. Total Colour Difference (ΔE)*

To examine the color shift in the sample that occurs while the ghee is being stored, the L*, a*, and b* values are insufficient. Color difference, often known as delta (Δ), is the result of comparing samples to a standard or starting value. It shows a variation in the absolute color coordinates. The overall color difference (ΔE*) is always positive, regardless of whether the deltas for L*, a*, and b* are positive or negative. Calculating the entire color difference was done using equation 3.6.

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad \text{..... (3.6)}$$

Where, ΔL* is the difference in lightness and darkness and its positive and negative value corresponds to lighter and darker respectively and Δa* is the difference in red and green its positive value corresponds to redder and negative value correspond to greener and Δb* is the difference in yellow and blue its positive difference corresponds to yellower and negative correspond to bluer.

Fig 5: Colour Analysis of Blended Ghee Samples using Chroma Meter

L. Statistical Analysis

All determination carried out in triplicate and data was analyzed with coefficient of variation, mean and standard deviation. The Microsoft Excel Data Analysis Tool was used for statistical analysis.

III. RESULTS AND DISCUSSION

An attempt has been made to solvent extraction of lycopene from tomato paste with an aim to use its antioxidant properties to enhance the shelf life of ghee or preservation of ghee. The following results are obtained during the project:

A. Extracted Yield of Lycopene

The extraction of red pigment lycopene from tomato was done using food grade solvent hexane. The 30 ml of hexane add into 100g of tomato paste and stirred on magnetic stirrer 4-5 minutes. After that decant the aqueous solution layer; this has to be done 4-5 times and kept in SOCS PLUS apparatus to evaporate the traces of solvent. The yield of lycopene pigment was 165.67 ± 1.20 mg/kg tomato paste and lycopene content in pigment was 67.82% of total pigment. This was calculated by equation 4.1.

B. Observation of UV- Visible Absorption Spectra of Lycopene

The lycopene in hexane UV-visible absorption spectrum was measured using a spectrophotometer (Epoch 2, BioTek, USA, 96 well microplate). The absorption spectra nearly coincided with the trans-lycopene maximum characteristic peak. These lycopene UV-visible spectra were contrasted with Welni U. et al.'s published study from 2001. A UV spectrophotometer was used to analyze the spectra of the extracted sample. Figure 6 shows the UV-visible spectrum of the lycopene concentration. The highest peak features of lycopene in hexane solution were found at 470 nm. Using equation 4.1, the sample's lycopene content was determined in percentage terms at its peak wavelength of 470 nm.

Lycopene (%) = (Absorbance at 472 nm)/ (3450×l×specimen weight(g))…….. (4.1)

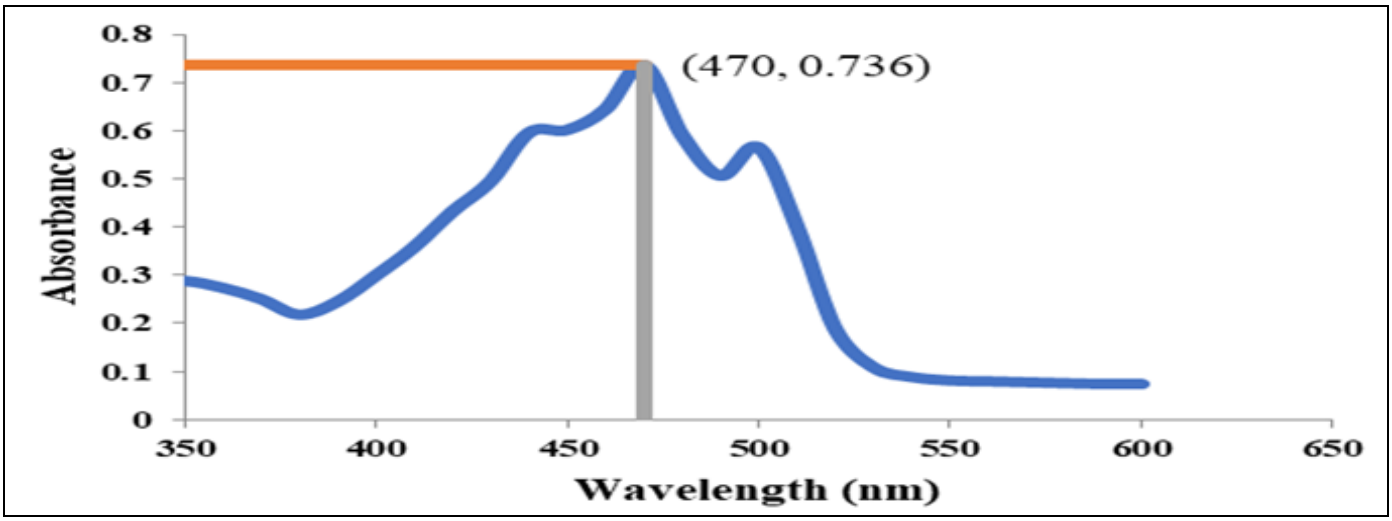

Fig 6: UV- Visible Absorption Spectra of Extracted Lycopene Pigment

C. Sensory Evaluation

Lycopene (LC) extracted from tomato was added in the ghee in different concentration (0.01%, 0.02%, 0.05%, 0.1%, 0.25% and 0.5% w/w). Sensory evaluation was done according to the 9-point hedonic scale for eight samples including ghee blended with Butylated hydroxyanisole (BHA).

Different samples correspond to: A – Control; B – Ghee+ LC (0.01% w/w); C – Ghee +LC (0.02% w/w); D – Ghee + LC (0.05% w/w); E – Ghee + LC (0.1% w/w); F – Ghee + LC (0.25% w/w); G – Ghee + LC (0.5% w/w); H – Ghee + BHA (0.02% w/w).

D. Determination of Total Antioxidant Capacity (TAC)

The findings of the phospomolybdnem technique, which was used to determine the total antioxidant capacity of the samples, are displayed. TAC, which is expressed as mmol α-tocopherol/g sample, was used to calculate the ultimate lycopene content between two samples (0.01% and 0.02%). TAC values were higher in the lycopene blended ghee (0.02%) than in BHA and the resulting lower lycopene content (0.01%). Thus, at a final concentration of 0.02%, lycopene and ghee were combined; other concentrations were not included.

E. Free Radical Scavenging Activity

When compared to alternative approaches, the concept of scavenging the stable DPPH radical is a popular way to assess antioxidant activity quickly (Gulcin, 2004). The lycopene's ability to scavenge is shown by the degree of dis-colouration. Ghee's unsaturated lipids auto-oxidize due to free radicals.

When assessing antioxidant activity quickly, the idea of scavenging the stable DPPH radical is a better option than alternative techniques (Gulcin, 2004). The degree of discolouration tells us about lycopene's scavenging ability.

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Ghee's unsaturated lipids auto-oxidize due to free radicals. It is clear that ghee infused with BHA and lycopene had radical scavenging activities of 75.25% and 68.05%, respectively, prior to heat treatment, while the control (unblended) ghee had 8.10%.

The percentage inhibition on 49th days was determined to be 0.86%, 27.45%, and 26.0% for unblended ghee, ghee blended with BHA, and ghee treated three times, respectively.

Table 2: Results Showing the Samples Inhibition in %

Samples	Inhibition (%)
Unblended Ghee	8.10
Ghee + 0.02% LC	68.05
Ghee+ 0.02% BHA	75.25

The coefficient of variation (CV) of the unblended ghee samples was higher as compared to ghee blended with 0.02% BHA and 0.02% lycopene.

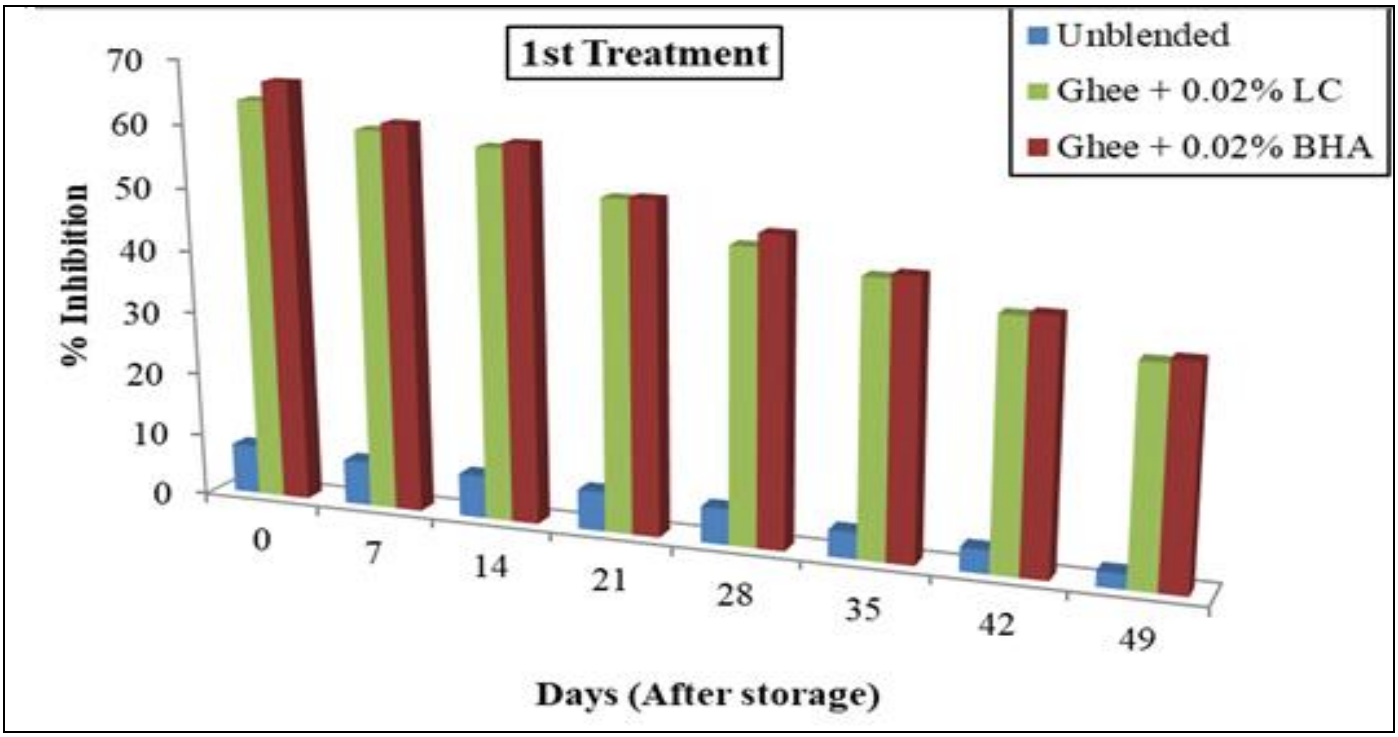

Fig 7: DPPH Radical Scavenging Activity of Various Ghee Samples after One Treatment

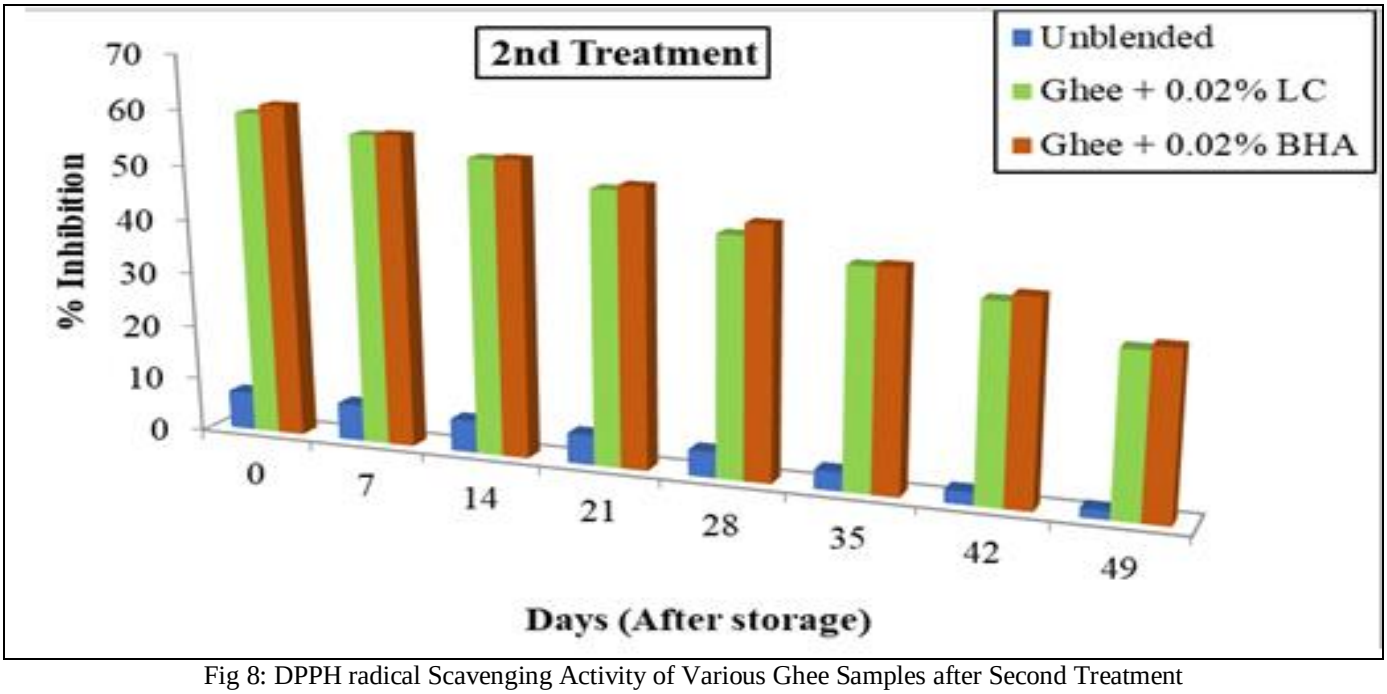

Fig 8: DPPH radical Scavenging Activity of Various Ghee Samples after Second Treatment

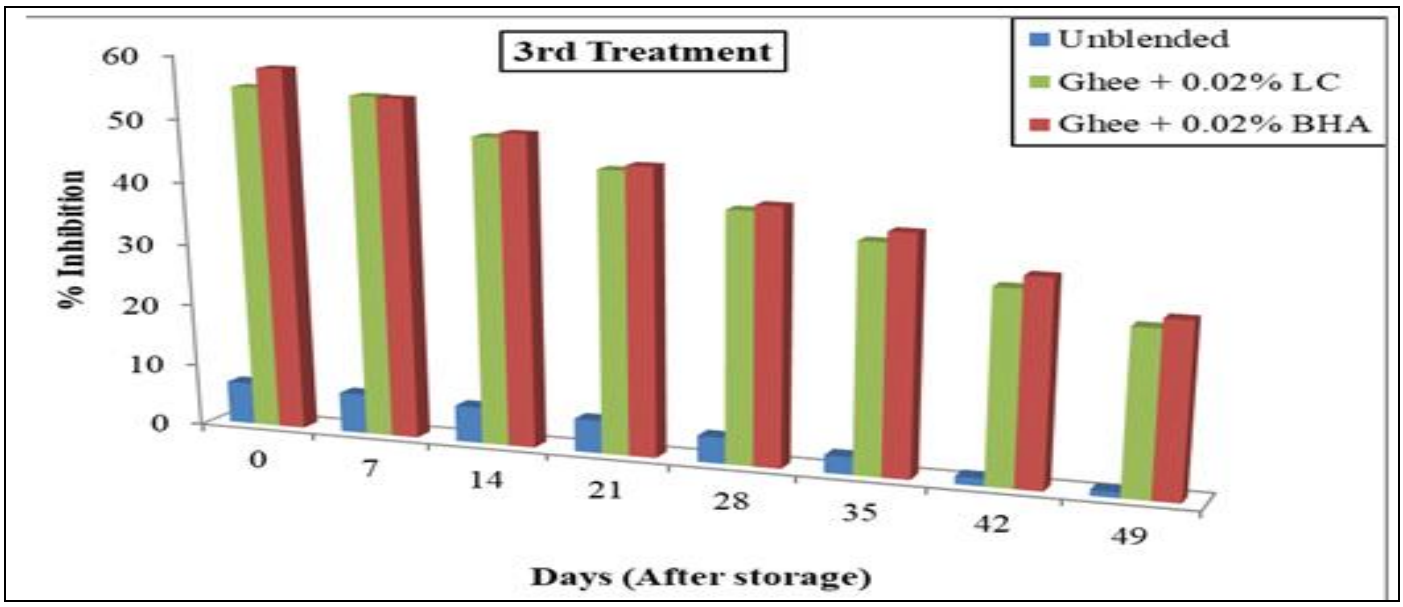

Fig 9: DPPH Radical Scavenging Activity of Various Ghee Samples after Third Treatment

F. Colour Measurement

Beginning at the start of the addition and continuing for 49 days of analysis, the L*, a*, and b* color tests were performed. The color values of the ghee samples were recorded as follows: L* (lightness), a* (redness), and b* (yellowness). As the ghee's blackness grew, the value of L* fell. Oxidation of ghee may be the reason. Redness, or a*, increased significantly from 0.96 to 4.20, yellowness, or b*, ranged from 18.99 to 9.01, and L* decreased from 62.05 to 39.89 in the first thermally treated unblended ghee. The blended ghee samples containing lycopene and BHA had a coefficient of variation of 10.61% and 11.17%, respectively, while the unblended ghee samples had a coefficient of variance of 16.91% and 17.70% for the second and third thermally treated blended ghee samples.

G. Total Colour Difference (ΔE)*

The coefficient of variation was used to examine the overall color difference between blended and unblended ghee following heat treatment. First, the ghee mixed with lycopene had a coefficient of variation of 54.16%, whereas the ghee blended with BHA and the unblended ghee had coefficients of variation of 56.81% and 54.16%, respectively. After the initial heat treatment, the combined ghee samples with lycopene and BHA showed no further alteration in overall color.

IV. SUMMARY AND CONCLUSION

Investigating the potential of tomatoes' inherent antioxidants for lipid or fat-containing food preservation is the aim of this study. A spectrophotometer's UV-visible absorption spectra were used to establish the identity of lycopene after it had been extracted using a solvent. A spectrophotometer's UV-visible absorption spectra were used to establish the identity of lycopene after it had been extracted using a solvent. The potential of lycopene as a naturally occurring antioxidant in ghee was examined in this study. Since lycopene is more stable under processing circumstances than other antioxidants, it may be used as one of the natural antioxidants even if it has not yet been studied or documented, particularly in relation to ghee.

Finally, it can be concluded that unblended ghee deteriorated faster than blended ghee samples with BHA (0.02%) and lycopene (0.02%) as apparent from the observation on total antioxidant capacity, colour values (L*, a* and b*), percentage inhibition, peroxide value and free fatty acid percent due to thermal treatments. As a result of thermal treatments, it is evident from the observations of total antioxidant capacity, color values (L*, a*, and b*), percentage inhibition, peroxide value, and free fatty acid percent that unblended ghee degraded more quickly than blended ghee samples containing BHA (0.02%) and lycopene (0.02%).

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