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RESEARCH ARTICLE

Efficacy of microencapsulated *galgal* essential oil as a natural preservative in anhydrous milk fat (*Ghee*)

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Abstract: The growing concern over health risks associated with synthetic preservatives has led to an increased demand for natural alternatives in food preservation. Ghee, a traditional dairy product, is widely used in various food formulations and requires effective preservation methods to enhance its shelf life. This study investigates the potential of galgal (*Citrus pseudolimon*) peel essential oil as a natural preservative for ghee. Galgal is an underutilized citrus fruit, and its peel is often considered waste. Essential oil was extracted from the peel and microencapsulated using spray drying technology with maltodextrin and gum Arabic (1:1) as wall materials, along with 1% soy lecithin. Fourier Transform Infrared (FTIR) Spectroscopy analysis confirmed the presence of key bioactive compounds, including limonene, α-pinene, β-pinene, and γ-terpinene, with characteristic peaks at 797 cm^{-1}, 887 cm^{-1}, 1375 cm^{-1}, 1643 cm^{-1}, and 2963 cm^{-1}. The microencapsulated powder was incorporated into ghee at concentrations of 0.5%, 1%, and 1.5% and compared with BHA (0.02%) over 180 days of storage at room temperature. The 1.5% powder addition was the most effective, as indicated by minimal increases in acid value (0.111 to 0.126 mg KOH/g), peroxide value (0.221 to 0.294 meq/kg), and p-anisidine value (0.10 to 0.17 at 350 nm). A slight reduction in iodine value was observed. Rancimat analysis further confirmed enhanced oxidative stability in ghee with 1.5% powder. These findings highlight the potential of microencapsulated galgal peel essential oil as a natural preservative to enhance ghee's shelf life.

Keywords: Galgal, essential oil, microencapsulation, antioxidant, ghee, oxidative stability

Introduction

Ghee, also known as clarified butterfat or anhydrous milk fat, is traditionally made from cow or buffalo milk. It is highly valued for its rich aroma, vibrant color, and ability to enhance the flavor of various dishes. With a fat content of at least 99.5%, ghee is considered one of the most versatile and effective cooking and frying mediums. However, its stability during storage is challenged by oxidative degradation, which can be influenced by several factors, including storage temperature (which determines whether ghee remains in a liquid or solid state), oxygen exposure (affected by packaging type and condition), and its physical state (Gandhi et al. 2018). Oxidative degradation deteriorates the color, flavor, aroma, and nutritional quality of ghee, leading to a decline in its shelf life and overall acceptability. The adverse effects of lipid oxidation are not limited to deteriorating food quality but also pose significant health concerns. Several studies (Mao et al. 2024; Zhou et al. 2025; Irisarri et al. 2025) have shown that oxidized lipids contribute to various health risks, including cancer, heart disease, and premature aging (Pramanik et al. 2023). To mitigate these risks and extend the shelf life of oils, the use of antioxidants is essential. Antioxidants help stabilize oils, prevent lipid oxidation, and protect against the harmful effects of oxidized products such as free radicals (Wang et al. 2024). Traditionally, synthetic antioxidants like butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), and butylated hydroxytoluene (BHT) have been widely used to delay oxidation and enhance food stability. However, concerns about their potential toxicity have led to regulatory restrictions in several countries due to their possible adverse effects on human health and the environment (Kohli et al. 2024). Although synthetic antioxidants are used in low concentrations, their long-term health implications cannot be ignored (Lalani et al. 2024). Consequently, there has been a growing interest in

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natural antioxidants, prompting extensive research into plant-based and other naturally derived compounds as safer alternatives (Rahaman et al. 2023; Girish et al. 2023).

Among natural sources, citrus fruits are one of India's most economically significant crops. The Citrus genus, which comprises approximately 140 genera and 1,300 species, belongs to the Rutaceae family. Within this genus, *Citrus pseudolimon*, commonly known as galgal, is an underutilized species with great potential (Grover et al. 2024a). One of the major challenges associated with citrus cultivation is the generation of substantial amounts of waste, which accounts for nearly 50% of the total fresh fruit mass (Grover et al. 2024b). Consumption and processing of citrus fruits result in large quantities of byproducts. Among these, citrus peels are particularly valuable as they contain essential oils (EOs), which can be extracted and utilized to manage agro-industrial waste efficiently. These essential oils are rich in bioactive compounds with antioxidant properties, making them suitable for extending the shelf life of food products (Darwish, 2024).

Beyond their antioxidant potential, essential oils have diverse applications across multiple industries, including food, cosmetics, pharmaceuticals, and aromatherapy. They are widely used as natural flavoring agents and fragrances in food and cosmetic formulations. The United States Food and Drug Administration (FDA) has classified essential oils as generally recognized as safe (GRAS), further supporting their potential as viable substitutes for synthetic additives (Grover et al. 2024). Given these benefits, essential oils from citrus peels present a promising natural alternative to synthetic antioxidants in food preservation. Building on this premise, the objective of the present study was to evaluate the effectiveness of microencapsulated galgal essential oil as a natural preservative in ghee. The microencapsulation process was employed to enhance the stability and controlled release of essential oil, making it more suitable for food applications. In this study, microencapsulated essential oil powder was incorporated into ghee at various concentrations to assess its impact on shelf stability. By analysing oxidative stability parameters over a storage period, the study aimed to determine whether microencapsulated galgal essential oil could serve as an effective natural preservative to prolong the shelf life of ghee while maintaining its quality attributes.

Material and Methods

Procurement of chemicals and reagents

Ethyl alcohol, NaOH, phenolphthalein indicator, BHA, maltodextrin, gum arabic, soy lecithin, acetic acid, chloroform, potassium iodide, starch, sodium thiosulfate, potassium dichromate, HCl, and Wij's solution, isooctane, p-anisidine solution were procured from Global Scientific Pvt. Ltd., Ludhiana. All chemicals used were of analytical reagent (AR) grade.

Extraction of essential oil from peel using various methods

Fully ripened galgal fruits were collected from the Fruit Research Farm of Punjab Agricultural University (PAU), Ludhiana, India. The fruits were peeled, and the peels were stored under refrigeration until further use. Prior to extraction, the peels were homogenized using a mixer grinder, and the resulting pulp was subjected to essential oil extraction using different techniques, including the Clevenger apparatus, steam distillation, microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE), and supercritical fluid extraction (SFE). In the MAE method, the homogenized peel was exposed to microwave radiation (2.45 GHz) for 10, 15, and 20 minutes. The essential oil was then extracted using a Clevenger apparatus, and the yield was calculated. Similarly, for UAE, the peel was treated with ultrasound for the same time intervals using a ultrasonic processor, operating at a frequency of 33+ kHz and a power of 80 watts. For SFE, the extraction was conducted at a temperature of 45°C and a pressure of 400 bar. The CO, flow rate was maintained at 5 mL/min for a total duration of 60 minutes, amounting to a total volume of 100 mL of CO, . To prevent clogging, the restrictor temperature was set 15°C higher than the extraction temperature. The yield of the extracted essential oil was determined using the gravimetric method.

Preparation of microencapsulated powder of essential oil

Maltodextrin (MD) and gum Arabic (GA) were dissolved in a 1:1 ratio in double-distilled water at 70°C, and soy lecithin was added at a concentration of 1%. The mixture was homogenized at 10,000 rpm for 10 minutes using a homogenizer. After cooling to room temperature, 20% essential oil was incorporated, followed by an additional 10-minute homogenization. The emulsion then underwent ultrasonic treatment for 15 minutes to enhance stability. The prepared emulsion was evaluated for emulsion stability and subjected to spray drying with a 0.5 mm diameter nozzle. The emulsion was fed through a peristaltic pump, with inlet and outlet temperatures set at 170°C and 80°C, respectively. The blower speed was maintained at 2,350 rpm, and the air pressure was set to 0.7 bar. The resulting powder was collected and stored in clean, airtight jars at room temperature for further analysis.

Characterization of bioactive components of essential oil powder by FTIR

The functional groups present in the essential oil were identified using FTIR spectroscopy. The analysis was performed using the attenuated total reflectance (ATR) technique on an Agilent Cary 630 instrument (USA) at a controlled temperature of 25°C. The ATR-FTIR spectrum was recorded within the wavenumber range of 4000-700 cm{ ¹. The obtained spectra were analyzed following the interpretation method outlined by Boughendjioua and Djeddi (2017).

Preparation of ghee

Ghee was prepared in the laboratory following the method described by Kumar et al. (2018). Fresh buffalo milk was procured from a local dairy farm in Ludhiana, boiled, and subsequently cooled to facilitate cream separation. The collected cream was churned to obtain butter, which was subsequently washed and purified. The butter was then heated in a pan at 108-110°C to facilitate the removal of moisture, converting it into ghee. After filtration to eliminate any impurities, the ghee was stored in PET jars for further analysis.

Preparation of ghee samples

Melted ghee (100 g) was measured into five separate beakers. The first beaker served as the control sample, while the second beaker contained 0.02% BHA. The remaining three beakers were supplemented with 0.5%, 1.0%, and 1.5% essential oil powder (EOP), respectively. Each sample was thoroughly mixed using a magnetic stirrer at 40°C for 20 minutes to ensure uniform distribution.

Storage of ghee samples

The prepared ghee samples were stored at room temperature (25±2°C) in PET jars, ensuring a clean and dry environment for the storage study.

Quality parameters of ghee

The quality parameters, including free fatty acid content, peroxide value, p-anisidine value, and iodine value, were analyzed according to the methods outlined in IS 548: Part 1: 1964 (2020).

Oxidative stability of ghee samples using Rancimat

The induction period was determined using a Metrohm Rancimat Model 743 (Herisau, Switzerland) at 140°C, following the method described by Tarmizi et al. (2016). A 0.3-gram oil sample was carefully placed at the base of the reaction tube. To ensure seamless operation, the conductivity measurement tube and air manifold were securely connected. The aeration tube was positioned within 5 mm of the bottom of both the reaction and conductivity tubes, and the airflow rate was adjusted to 2.5 ± 0.2 mL/s. A multi-channel strip chart recorder continuously monitored and recorded water conductivity data over time. The precise moment of significant oxidation changes was determined using a microprocessor-based slope technique. The induction period, expressed in hours, represents the time at which rapid oxidation begins under the specified conditions.

Statistical analysis

The experiments were conducted in triplicate, and the resulting data was presented as means ± standard deviation (SD).

Results and Discussion

Extraction & yield of essential oil

The extraction method plays a crucial role in determining the yield and composition of essential oils. This study evaluated different extraction techniques, including supercritical CO, extraction (SFE), Clevenger apparatus extraction, steam distillation, microwave-assisted extraction (MAE), and ultrasonic-assisted extraction (UAE), to identify the most efficient method for extracting essential oil from peel (Table 1). Among these, supercritical CO, extraction was found to be the most effective, yielding the highest percentage of essential oil 10.51±0.02%. However, this method is expensive due to its high operational and maintenance costs. The Clevenger extraction method emerged as the most economical technique, yielding 4.54±0.01%, while steam distillation was the least efficient, with yields as low as 1.80±0.05%. Among MAE and UAE, the MAE (20 min) method produced the highest yield, with 2.59±0.05% surpassing UAE in efficiency. Extraction methods significantly influence the composition of essential oils, impacting their potential applications in food, cosmetics, and pharmaceuticals (Grover et al, 2023).

Among the methods tested, supercritical CO, extraction gave the highest oil yield, while the Clevenger method emerged as the most cost-effective. Therefore, the final extraction of essential oil was carried out using the Clevenger method.

Characterization of bioactive components in microencapsulated essential oil

Fourier-transform infrared (FTIR) spectroscopy is widely used to identify functional groups. This study presents the infrared spectra of microencapsulated essential oil powder within the 4000-700 cm{ ¹ range (Figure 1). The results indicate a diverse mixture of organic compounds, each characterized by distinct vibrational bands.

Aromatic compounds were identified by strong C=C and C-H stretching vibrations at 797.65 cm{ ¹ and 887.11 cm{ ¹. Terpenoid components exhibited C-H bending at 987.74 cm{ ¹ and C-O stretching at 1107.02 cm{ ¹, 1148.02 cm{ ¹, 1151.75 cm{ ¹, and 1177.84 cm{ ¹. Alcohol and phenol groups were detected via O-H bending at 760.38 cm{ ¹ and 1375.39 cm{ ¹. Cyclohexane vibrations, linked to α-pinene, appeared at 1013.84 cm{ ¹ and 1017.56 cm{ ¹. C-N amine groups were observed at 1230.02 cm{ ¹ and 1274.75 cm{ ¹, while C=O stretching was noted at 1736.94 cm{ ¹ and 1770.49 cm{ ¹. Asymmetrical CH, and CH*f* stretching of alcoholic components were seen at 2963.23 cm{ ¹, with additional -C-H asymmetric and symmetric methylene stretch at 2918.51 cm{ ¹. The -OH stretch of linalool appeared at 3421.70 cm{ ¹.

Major peaks were found at 797 cm{ ¹, 887 cm{ ¹, 1375 cm{ ¹, 1435 cm{ ¹, 1643 cm{ ¹, and 2963 cm{ ¹, with minor peaks supporting C-

H, C=O, and C-O stretching of terpenoid components (Hasani et al. 2018). The peak at 2963 cm{ ¹ corresponds to asymmetrical CH, and CH*f* groups in alcoholic components (Berechet et al. 2015). Peaks at 2918 cm{ ¹, 1677 cm{ ¹, 1643 cm{ ¹, 1438 cm{ ¹, 1375 cm{ ¹, 1151 cm{ ¹, 887 cm{ ¹, and 797 cm{ ¹ were linked to alkane C-H stretching, C=C stretching, phenol O-H bending, tertiary alcohol C-O stretching, and aromatic C-H vibrations (Berechet et al. 2015; Benoudjit et al. 2020). The peaks at 1643 cm{ ¹, 887 cm{ ¹, and 797 cm{ ¹ confirm the presence of limonene, α-pinene, β-pinene, and γ-terpinenes. Similar study by Nunes et al. (2021) identified these peaks in lemon and galgal essential oils, respectively, further supporting the findings. The bioactive components from galgal essential oil such as α-Pinene, D-Limonene, Linalool, Terpinene, α-Phellandrene, Camphene, α-Myrcene, α-Terpineol, Caryophyllene, cis-α-Bergamotene, Citral etc were detected in our previous study (Grover et al. 2023) with the help of gas chromatography mass spectroscopy (GCMS). FTIR also revealed that in microencapsulated powder these components were present.

Storage study of ghee samples using various parameters

Acid Value

Table 2 presents the acid values of ghee samples stored for 180 days, comparing a control sample with ghee treated with 0.02% BHA and varying concentrations of Essential Oil Powder (EOP) at 0.5%, 1.0%, and 1.5%. The control sample showed a significant rise in acid value from 0.112% on day 0 to 0.268% at 180 days, indicating ongoing lipid hydrolysis and quality deterioration. In contrast, ghee treated with 0.02% BHA exhibited a much slower increase, from 0.111% to 0.118%, demonstrating its effectiveness in reducing hydrolytic rancidity. Similarly, EOP-treated samples had lower acid values than the control, with stability improving at higher concentrations. The 0.5% EOP-treated ghee showed an acid value increase from 0.112% to 0.130%, while the 1.0% EOP sample rose from 0.112% to 0.129%. The 1.5% EOP-treated ghee exhibited the highest stability, with an increase from 0.111% to 0.126% over 180 days. These results indicate that both BHA and EOP effectively inhibited free fatty acid formation, delaying hydrolytic degradation. Among the treatments, higher concentrations of EOP (1.5%) provided the best stability, comparable to BHA, highlighting the potential of both natural (EOP) and synthetic (BHA) antioxidants in enhancing ghee's storage stability.

Table 1: Yield of extracted essential oil using various methods

Technique used	Time (minutes)	% Yield of EO
Clevenger	60	04.54 ± 0.01^{b}
Steam Distillation	80	01.80 ± 0.05^{e}
MAE for 10 min	50	02.41 ± 0.04^{d}
MAE for 15 min	50	02.43 ± 0.02^{d}
MAE for 20 min	47	02.59 ± 0.05^{cd}
UAE for 10 min	49	02.62 ± 0.03^{cd}
UAE for 15 min	46	02.45 ± 0.04^{d}
UAE for 20 min	41	02.48 ± 0.06^{d}
Supercritical CO_2	70	10.51 ± 0.02^{a}

Values are means of triplicate ± standard deviation
Within a column with the same lowercase letter are not significantly different at $p<0.05$

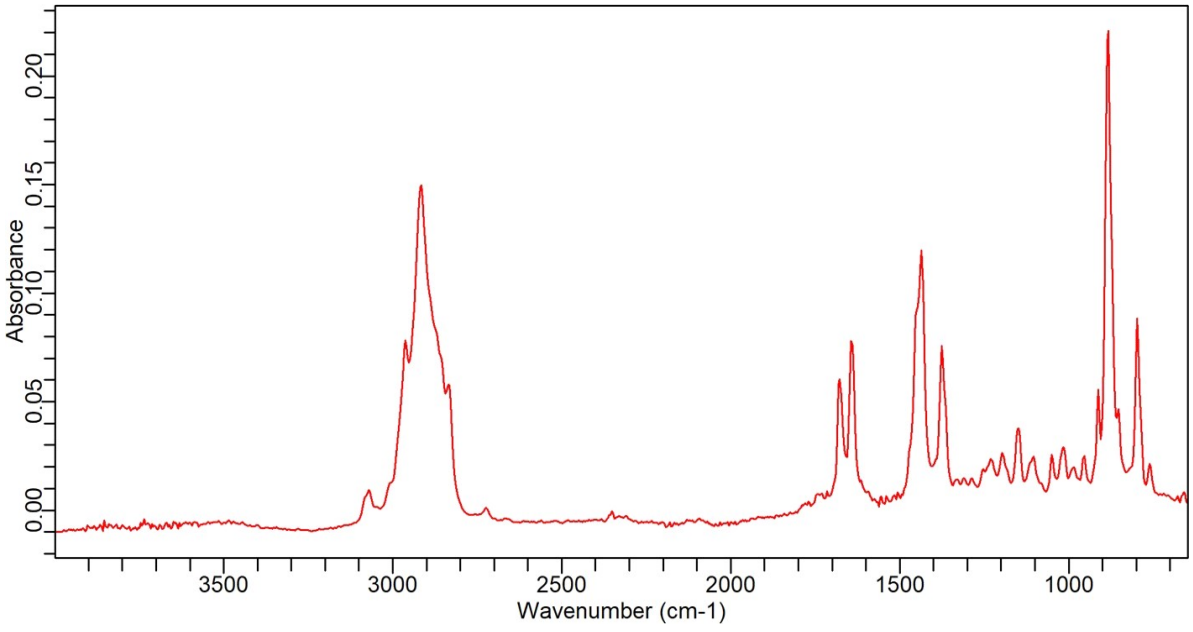

Fig. 1 FTIR spectra of essential oil powder

Prolonged storage increases acid values in fats due to triglyceride hydrolysis, leading to free fatty acid formation and quality deterioration (Pena-Serna & Restreop-Betancur, 2020). The control ghee sample showed a rise in acid values over 180 days, confirming its susceptibility to rancidity (Nekera et al. 2023). In contrast, EOP-treated samples exhibited lower acid values, supporting findings by Kapadiya & Aparnathi (2018) on the antioxidant potential of essential oils in reducing lipid oxidation and extending shelf life.

Peroxide value

Table 2 presents the peroxide values (meqO, /kg) of ghee samples over 180 days, comparing untreated control ghee with samples treated with antioxidants: 0.02% BHA and Essential Oil Powder (EOP) at concentrations of 0.5%, 1.0%, and 1.5%. The control ghee sample showed a continuous increase in peroxide values, from 0.220 meqO, /kg on day 0 to 0.505 meqO, /kg by day 180, indicating significant oxidative degradation. This rise reflects

Table 2: Effects on quality parameters of ghee samples during various storage periods

Sample	Storage (Days)	Acid Value (%)	Peroxide Value (meqO2/kg)	p-anisidine Value	Iodine Value
Ghee (control)	0	0.112 ± 0.001a	0.220 ± 0.001a	0.10 ± 0.10a	38.66 ± 0.10a
	30	0.113 ± 0.001a	0.232 ± 0.001a	0.11 ± 0.01a	38.65 ± 0.11a
	60	0.115 ± 0.001a	0.240 ± 0.001a	0.13 ± 0.01a	38.61 ± 0.10a
	90	0.120 ± 0.002b	0.295 ± 0.001b	0.15 ± 0.02b	38.60 ± 0.10a
	120	0.155 ± 0.001b	0.324 ± 0.001b	0.18 ± 0.01b	38.55 ± 0.11a
	150	0.165 ± 0.002b	0.402 ± 0.001c	0.19 ± 0.02c	38.52 ± 0.10a
	180	0.268 ± 0.002c	0.505 ± 0.001c	0.25 ± 0.02c	38.43 ± 0.10a
Ghee (0.02% BHA)	0	0.111 ± 0.002a	0.220 ± 0.002a	0.10 ± 0.01a	38.66 ± 0.11a
	30	0.111 ± 0.001a	0.225 ± 0.001a	0.10 ± 0.01a	38.66 ± 0.11a
	60	0.112 ± 0.002a	0.231 ± 0.002a	0.10 ± 0.01a	38.65 ± 0.10a
	90	0.113 ± 0.002ab	0.240 ± 0.001ab	0.11 ± 0.01ab	38.65 ± 0.10a
	120	0.115 ± 0.001b	0.258 ± 0.001b	0.11 ± 0.02ab	38.64 ± 0.11a
	150	0.116 ± 0.002b	0.271 ± 0.002bc	0.12 ± 0.02b	38.62 ± 0.10a
	180	0.118 ± 0.001c	0.288 ± 0.001c	0.13 ± 0.02c	38.60 ± 0.10a
Ghee (0.5% EOP)	0	0.112 ± 0.001a	0.220 ± 0.002a	0.10 ± 0.02a	38.65 ± 0.10f
	30	0.112 ± 0.001a	0.224 ± 0.002a	0.10 ± 0.01a	38.66 ± 0.11f
	60	0.113 ± 0.002ab	0.242 ± 0.002b	0.12 ± 0.02b	38.64 ± 0.10e
	90	0.116 ± 0.001b	0.271 ± 0.002c	0.14 ± 0.01c	38.60 ± 0.12d
	120	0.120 ± 0.002c	0.287 ± 0.002d	0.15 ± 0.02cd	38.57 ± 0.12c
	150	0.125 ± 0.001d	0.297 ± 0.001d	0.18 ± 0.01de	38.55 ± 0.11b
	180	0.130 ± 0.002e	0.329 ± 0.001e	0.20 ± 0.02e	38.50 ± 0.10a
Ghee (1.0% EOP)	0	0.112 ± 0.002a	0.220 ± 0.001a	0.10 ± 0.02a	38.66 ± 0.11e
	30	0.112 ± 0.001a	0.223 ± 0.001a	0.11 ± 0.02a	38.66 ± 0.11e
	60	0.113 ± 0.001a	0.233 ± 0.002b	0.11 ± 0.01a	38.65 ± 0.10de
	90	0.115 ± 0.002ab	0.268 ± 0.002c	0.12 ± 0.01ab	38.64 ± 0.10d
	120	0.117 ± 0.001b	0.280 ± 0.001d	0.14 ± 0.01b	38.62 ± 0.11c
	150	0.124 ± 0.001c	0.293 ± 0.002d	0.16 ± 0.02c	38.60 ± 0.11b
	180	0.129 ± 0.002d	0.304 ± 0.002e	0.17 ± 0.03c	38.58 ± 0.10a
Ghee (1.5% EOP)	0	0.111 ± 0.001a	0.221 ± 0.001a	0.10 ± 0.01a	38.65 ± 0.11d
	30	0.111 ± 0.002a	0.222 ± 0.002a	0.10 ± 0.02a	38.65 ± 0.10d
	60	0.112 ± 0.002a	0.232 ± 0.001b	0.11 ± 0.02a	38.64 ± 0.11cd
	90	0.116 ± 0.001b	0.252 ± 0.001c	0.12 ± 0.02ab	38.64 ± 0.10cd
	120	0.118 ± 0.001bc	0.271 ± 0.002d	0.13 ± 0.02b	38.63 ± 0.10c
	150	0.123 ± 0.002cd	0.281 ± 0.001e	0.15 ± 0.02c	38.62 ± 0.10b
	180	0.126 ± 0.001d	0.294 ± 0.001f	0.17 ± 0.02d	38.59 ± 0.11a

Within a column with the same lowercase letter are not significantly different at $p<0.05$
Values are means of triplicate ± standard deviation.
BHA- Butylated hydroxyanisole
EOP- Essential Oil Powder

Fig. 2 Rancimat analysis of ghee samples for checking storage stability (induction period)

ongoing lipid oxidation, which negatively impacts ghee quality. In contrast, ghee treated with 0.02% BHA exhibited a much slower increase in peroxide values, from 0.220 meqO, /kg initially to 0.288 meqO, /kg at 180 days, demonstrating BHA's effectiveness in minimizing oxidation. Similarly, EOP-treated ghee samples showed lower peroxide values compared to the control. The 0.5% EOP-treated sample increased from 0.220 meqO, /kg to 0.329 meqO, /kg over 180 days, while the 1.0% EOP sample rose to only 0.304 meqO, /kg, indicating improved oxidative stability. The 1.5% EOP-treated ghee exhibited the lowest peroxide value increase, reaching just 0.294 meqO, /kg at 180 days. These results suggest that both BHA and EOP, particularly at higher concentrations, effectively slow down lipid oxidation and enhance ghee's oxidative stability. The dose-dependent response of EOP highlights its potential as a natural antioxidant alternative to synthetic additives like BHA for extending ghee's shelf life.

Studies have shown that essential oils possess strong antioxidant properties, making them effective natural preservatives (Tit & Bungau, 2023; Mrabti et al. 2023). Their bioactive compounds, including phenolics, terpenes, and flavonoids, help reduce oxidative stress and prevent spoilage, offering a natural alternative to synthetic preservatives. These findings support the potential of essential oils in enhancing food shelf life.

p-Anisidine value

Table 2 presents the p-anisidine values of ghee samples with different antioxidant treatments over 180 days. The control ghee sample exhibited a gradual increase in p-anisidine value from 0.10 ± 0.01 on day 0 to 0.25 by day 180, indicating progressive oxidative degradation. The ghee treated with 0.02% BHA showed the highest stability, with values remaining relatively steady, increasing only from 0.10 ± 0.01 at day 0 to 0.13 ± 0.02 at day 180, demonstrating effective inhibition of secondary oxidation. The samples treated with Essential Oil Powder (EOP) at 0.5%, 1%, and 1.5% exhibited intermediate stability compared to the control. The 0.5% EOP-treated ghee showed an increase in p-anisidine value from 0.10 ± 0.02 initially to 0.20 ± 0.02 at day 180, providing moderate oxidation protection. Similarly, the 1% EOP-treated sample showed a rise from 0.10 to 0.17, while the 1.5% EOP-treated ghee maintained a comparable trend, increasing from 0.10 to 0.17 by the end of the storage period. These findings indicate that while BHA was more effective in minimizing oxidative changes, EOP also significantly slowed the oxidation rate compared to the control. Higher concentrations of EOP generally provided better oxidative stability, demonstrating its potential as a natural antioxidant for prolonging ghee shelf life.

Natural antioxidants, especially essential oil powders (EOPs), are gaining popularity as clean-label alternatives to synthetic preservatives (Aguiar Campolina et al. 2023). Studies have shown that essential oils from plants like rosemary, oregano, and clove exhibit strong antioxidant properties due to their high phenolic content. Rosemary essential oil, for instance, effectively inhibits lipid oxidation, comparable to synthetic antioxidants like BHA and BHT (Song et al. 2023). Research also highlights a dose-dependent effect, where higher concentrations enhance protection, though efficacy varies based on fat type and storage conditions (Bayram & Decker., 2023). Investigations into mechanisms like free radical scavenging and metal ion chelation further support the potential of essential oils as sustainable food preservatives (Kohli et al. 2024).

Iodine value

Table 2 shows that the iodine value of ghee remained relatively stable throughout the 180-day storage period across all samples.

The control sample exhibited a slight decrease from 38.66 ± 0.10 at day 0 to 38.43 at day 180, indicating minimal changes in unsaturation levels. Similarly, ghee treated with 0.02% BHA showed negligible fluctuation, maintaining an iodine value of 38.66 at day 0 and 38.60 at day 180. The 0.5% EOP-treated sample displayed a minor decline from 38.65 ± 0.10 to 38.50 over the storage period. Likewise, the 1.0% and 1.5% EOP-treated ghee samples remained stable, with iodine values slightly decreasing from 38.66 at day 0 to 38.58 and 38.59 at day 180, respectively.

The stable iodine values observed across all ghee samples suggest that both synthetic (BHA) and natural (EOP) antioxidants effectively maintained the degree of unsaturation over the 180-day storage period, preventing significant oxidation or degradation of fatty acids. The minimal decline in iodine values indicates that antioxidant addition did not alter the lipid composition but played a crucial role in preserving ghee's overall stability. Typically, iodine values decrease during prolonged storage due to oxidative breakdown of unsaturated fatty acids (Omozuwa et al. 2023). However, the presence of antioxidants mitigated this degradation, as seen in the treated samples. Studies have demonstrated that synthetic antioxidants like BHA and natural additives such as essential oils can effectively stabilize oils by reducing oxidative stress (Samira et al. 2024). Additionally, research by Konfo et al. (2023) highlights the strong antioxidant properties of essential oils in maintaining the oxidative stability of fats and oils, particularly under extended storage conditions. These findings support the use of both synthetic and natural antioxidants in preserving ghee's quality and extending its shelf life.

Oxidative stability of ghee samples using Rancimat

The oxidative stability of ghee samples was evaluated using the Rancimat instrument, with induction time serving as a key indicator of antioxidant effectiveness. The induction time, also known as the oxidative stability index, represents the duration required for oxidation to reach a critical point, typically marked by a rapid increase in oxidation rate or the onset of rancidity. Figure 2 illustrates the Rancimat analysis of ghee samples, highlighting the impact of antioxidants on oxidative stability. The control ghee, without any antioxidants, exhibited the shortest induction time of 13.6 hours, indicating its high susceptibility to oxidation. In contrast, ghee treated with 0.02% BHA demonstrated the longest induction time of 22.5 hours, reflecting superior oxidative stability. Ghee samples supplemented with EOP at 0.5%, 1%, and 1.5% showed progressive improvements in stability, with induction times of 15.2, 16.5, and 17.4 hours, respectively. While EOP significantly enhanced oxidative stability, its effect was not as pronounced as that of BHA.

The longer induction times in antioxidant-treated samples confirm the effectiveness of these additives in delaying oxidation, thereby improving ghee's overall stability and shelf life. The shorter induction time of the control sample further emphasizes the vulnerability of ghee to oxidative deterioration in the absence of antioxidants. Previous studies have also demonstrated that essential oils and natural extracts possess strong antioxidant properties, positively influencing the shelf life of ghee (Pajohi et al. 2020; Kapadiya & Aparnathi., 2018). These findings support the role of both synthetic and natural antioxidants in preserving the quality of edible fats and oils during storage.

Conclusion

This study demonstrated the effectiveness of microencapsulated essential oil powder (EOP) and synthetic antioxidant BHA in enhancing the oxidative and hydrolytic stability of ghee during storage. FTIR analysis confirmed that bioactive compounds such as terpenes and phenols contributed to the antioxidant activity of EOP. The significant reduction in free fatty acid and peroxide formation in ghee treated with both BHA and EOP extended its oxidative stability beyond 180 days. Predictive statistical analysis indicated that EOP, particularly at higher concentrations, could serve as a viable natural alternative to BHA. Furthermore, the stable iodine values and prolonged induction times in Rancimat tests suggested that antioxidant treatment did not alter the lipid composition but effectively improved resistance to oxidation. These findings highlight the potential of EOP as a natural preservative for ghee and other edible oils, aligning with the increasing demand for clean-label, chemical-free food products. Future research should explore the long-term health implications of consuming EOP-treated ghee, considering the bioactive properties of essential oils. Additionally, evaluating the impact of various storage conditions on EOP efficacy and expanding its application to other oxidation-prone food products could provide valuable insights for the broader food industry. Understanding consumer acceptance and market viability of EOP-treated ghee would further support its adoption as a sustainable and natural preservation solution.

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