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Fourier-Transform Infrared (FTIR) Spectroscopy Evidence of Enhanced Functionality in Butter Fortified with Silibinin-Oleate

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Abstract
Butter is a fat-rich dairy product highly prone to lipid oxidation, resulting in quality deterioration and reduced shelf life. Although synthetic antioxidants such as BHT are effective, current food science trends favor natural or nature-derived alternatives. Silibinin, a flavonolignan from *Silybum marianum*, exhibits strong antioxidant properties but limited lipophilicity, restricting its use in lipid-based foods. To address this limitation, a lipophilic derivative, silibinin-oleate (SIL-O), was evaluated as a functional antioxidant for butter fortification. Butter samples were enriched with SIL-O at 100, 200, and 300 ppm, while butter without antioxidants and butter containing 200 ppm BHT served as controls. Molecular interactions between SIL-O and the butter lipid matrix were investigated using ATR-FTIR spectroscopy. The results showed concentration-dependent spectral changes in SIL-O-fortified samples, indicating effective incorporation into the lipid matrix and preservation of the antioxidant flavonolignan structure. In contrast, BHT produced only minor spectral modifications. These findings highlight SIL-O as a promising bioactive ingredient for functional butter development.
Key words: lipid oxidation, natural antioxidants, silibinin-oleate

1. Introduction

Butter is a widely consumed dairy product valued for its sensory attributes and high energy density; however, its elevated fat content, rich in saturated and unsaturated fatty acids, makes it particularly susceptible to lipid oxidation during processing and storage. Oxidative degradation of milk fat leads to the formation of hydroperoxides and secondary oxidation products, resulting in off-flavors, nutritional losses, and reduced shelf life [1]. To mitigate these effects, antioxidants are commonly employed, with synthetic compounds such as butylated hydroxytoluene (BHT) being widely used due to their effectiveness and low cost. Nevertheless, growing consumer demand for clean-label foods and increasing concerns regarding the long-term safety of synthetic additives have driven research toward natural or nature-derived alternatives [2].

In this context, polyphenols and flavonoid-based compounds have attracted considerable attention owing to their strong antioxidant activity and additional health-promoting properties. Among these, silibinin—the major bioactive constituent of silymarin extracted from *Silybum marianum*—has been extensively studied for its antioxidant, hepatoprotective, anti-inflammatory, and cytoprotective effects [3,4]. Despite its promising biological activity, the application of silibinin in fat-

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rich food systems remains limited due to its relatively low lipophilicity and poor solubility in nonpolar matrices.

To overcome these limitations, recent studies have focused on the chemical modification of silibinin through esterification with fatty acids, yielding lipophilic derivatives with improved compatibility in lipid environments. Silibinin-oleate (SIL-O) represents a particularly promising derivative, as the oleate moiety enhances solubility in lipid matrices while preserving the functional flavonolignan core responsible for antioxidant activity [5]. Such structural modification aligns with the broader concept of "lipophilization" of polyphenols, which has been shown to enhance antioxidant efficiency, stability, and distribution in lipid-based foods [6].

From a functional food perspective, incorporating SIL-O into butter offers a dual advantage: (i) improved oxidative stability of the lipid matrix, and (ii) enrichment of the product with a bioactive compound of documented physiological relevance. This approach goes beyond the conventional role of antioxidants as purely technological additives and supports the development of butter as a functional dairy product with added nutritional value. However, the successful implementation of such fortification strategies requires not only oxidative stability assessment but also molecular-level confirmation of the interaction between the bioactive compound and the fat matrix.

Fourier-transform infrared (FTIR) spectroscopy has emerged as a rapid, non-destructive analytical tool for investigating lipid structure, oxidation phenomena, and the incorporation of bioactive compounds in complex food systems. FTIR analysis enables the identification of characteristic functional groups and provides insights into molecular interactions through changes in band intensity, position, and shape [7]. In dairy fats, FTIR has been successfully applied to monitor lipid oxidation, detect antioxidant effects, and differentiate between native and modified lipid structures [8]. Despite these advantages, limited information is available regarding the FTIR spectral behavior of butter fortified with lipophilic derivatives of flavonoids such as silibinin-oleate.

Therefore, the aim of the present study was to investigate the molecular and functional implications of butter fortification with silibinin-oleate at different concentration levels (100–300 ppm), in comparison with a control sample and butter containing 200 ppm BHT. FTIR spectroscopy was employed to elucidate structural changes in the lipid matrix associated with antioxidant incorporation and to provide spectroscopic evidence supporting the enhanced functionality of SIL-O-fortified butter. The findings contribute to the growing body of research on natural antioxidant strategies and support the development of innovative functional dairy products.

2. Materials and Methods

2.1. Materials

Sweet cream butter (82% fat, unsalted) was obtained from a local dairy processor and used as the lipid matrix for all experimental samples. Silibinin-oleate (SIL-O), a lipophilic ester derivative of silibinin, was synthesized and purified according to previously reported procedures based on fatty acid esterification of flavonoids [9]. The purity of SIL-O was ≥95%, as confirmed by chromatographic analysis. Butylated hydroxytoluene (BHT, ≥99% purity) was purchased from a certified chemical supplier and used as a reference synthetic antioxidant. All solvents and reagents used were of analytical or HPLC grade.

2.2. Preparation of butter samples

Butter was gently melted in a water bath at 40–45 °C to obtain a homogeneous lipid phase, avoiding thermal degradation. SIL-O was first dissolved in a small amount of melted butter to ensure complete dispersion and then incorporated into the bulk butter to obtain final concentrations of 100 ppm (S1), 200 ppm (S2), and 300 ppm (S3), expressed on a fat basis. The mixtures were homogenized using a high-speed laboratory homogenizer (10,000 rpm, 2 min) under reduced light exposure to prevent photo-oxidation.

A butter sample containing 200 ppm BHT was prepared following the same procedure and served as a reference antioxidant system. Control butter was processed identically but without antioxidant addition. All samples were poured into opaque containers, flushed with nitrogen, sealed, and stored at 4 ± 1 °C until analysis.

2.3. FTIR spectroscopy analysis

Fourier-transform infrared (FTIR) spectra were recorded using an FTIR spectrometer Nicolet Is50 FT-IR (Thermo Fisher Scientific, Massachusetts, USA) equipped with an attenuated total reflectance (ATR) accessory. Butter samples were equilibrated to room temperature (22 ± 2 °C) prior to analysis to ensure consistent spectral acquisition. Approximately 20 mg of each sample was placed

directly onto the ATR crystal.
Spectra were collected in the mid-infrared region from 4000 to 650 cm^{-1}, with a resolution of 4 cm^{-1} and 64 scans per sample. A background spectrum was recorded before each measurement and automatically subtracted. Each sample was analyzed in triplicate, and the resulting spectra were averaged to minimize instrumental noise [9].
Spectral data were baseline-corrected and normalized prior to comparative analysis. Key spectral regions associated with lipid structure and antioxidant incorporation were evaluated, including:
(i) 3000–2800 cm^{-1} (aliphatic C–H stretching),
(ii) 1750–1700 cm^{-1} (ester carbonyl C=O stretching),
(iii) 1650–1600 cm^{-1} (aromatic C=C stretching), and
(iv) 1200–1000 cm^{-1} (C–O and C–O–C stretching vibrations).

2.4. Data analysis and interpretation

Differences among spectra were assessed qualitatively through band position, shape, and intensity variations, and semi-quantitatively by comparing relative absorbance changes in selected spectral regions. Particular attention was given to concentration-dependent spectral modifications induced by SIL-O incorporation, in comparison with control butter and the BHT-containing sample.
Spectral interpretations were performed by correlating observed FTIR features with known vibrational assignments of triglycerides, fatty acids, and flavonoid-derived compounds, as reported in the literature [7,8]. The FTIR results were further discussed in relation to the functional role of SIL-O as a lipophilic antioxidant and its potential contribution to enhanced oxidative stability and functionality.

2.5. Statistical considerations

While FTIR spectroscopy was primarily employed as a qualitative and comparative tool, consistency across replicates was ensured to support the reliability of spectral trends. Where applicable, relative intensity differences were used to support concentration-dependent interpretations.

3. Results and discussions

Analysis of FTIR spectra (Figure 1) highlights that the fortification of butter with silibinin-oleate (SIL-O) is not limited to a simple addition of antioxidant, but causes coherent, concentration-dependent spectroscopic changes, reflecting an efficient molecular integration of the bioactive compound into the lipid matrix. These changes support the concept of functional butter, with superior antioxidant potential and improved oxidative stability.

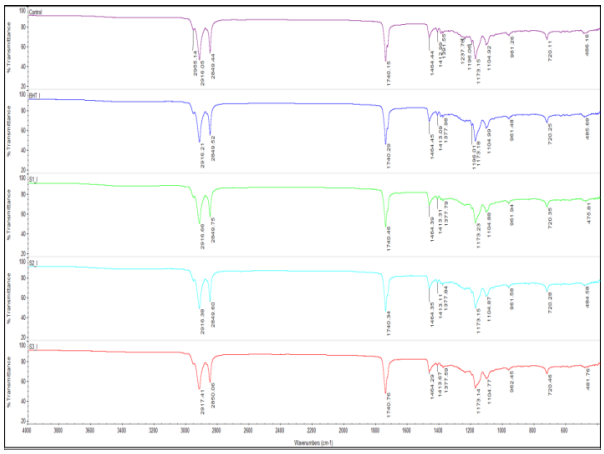

Figure 1. FTIR-spectra of butter samples C0- control sample (purple line), BHT spectra of butter sample with 200 ppm BHT (dark blue line), S1_1 butter sample with 100 ppm SIL-O (green line), S2_1 butter sample with 200 ppm SIL-O (blue line) and S3_1 butter sample with 300 ppm SIL-O (red line). Spectral range of 4000-400 cm^{-1}, 32 scans at 4 cm^{-1} resolution.

The FTIR spectra of the analyzed samples show the characteristic bands of the lipid matrix of butter, dominated by vibrations specific to triglycerides, saturated and unsaturated fatty acids, as well as the minor fraction of water and bioactive compounds. However, the addition of antioxidants (BHT or silibinin-oleate – SIL-O) induces subtle but systematic changes in the intensity and position of some bands, reflecting different molecular interactions between antioxidants and the fat phase.
First differences appear in the 3000–2800 cm^{-1} range characteristics for aliphatic C–H stretching vibrations.
In the Control sample, the intense bands at approximately 2922 cm^{-1} (ν_as CH_2) and 2852 cm^{-1} (ν_s CH_2) are typical of long aliphatic chains of fatty acids in triglycerides. The intensity of these bands is relatively constant, indicating an undisturbed lipid structure.
For the 200 ppm BHT sample, a slight increase in the relative intensity of the CH_2 bands is observed, without significant position shifts. This behavior is consistent with the structure of BHT, a bulky phenolic antioxidant, which dissolves in the fat phase, but does not specifically interact with lipid chains, its effect being predominantly radical-

scavenger and not structural. The FTIR literature reports minimal effects of BHT on the CH_2 bands of triglycerides, even at concentrations of the order of hundreds of ppm [10].

In samples S1, S2 and S3 (SIL-O 100–300 ppm), a progressive increase in the intensity of the CH_2 bands is observed, correlated with the SIL-O concentration. This trend suggests a more efficient integration of SIL-O into the lipid matrix, due to the oleate chain, which presents high structural compatibility with the fatty acids in butter. Recent studies on lipophilic derivatives of silibinin show that esterification with fatty acids favors hydrophobic interactions and stabilization of bioactive compounds in lipid environments.

The progressive increase in the intensity of the bands characteristic of aliphatic chains (2922 and 2852 cm^{-1}) in samples S1–S3 indicates that SIL-O, due to the oleate chain, exhibits a high affinity for the fat phase of butter. This structural compatibility is essential for functionality, since the efficiency of lipophilic antioxidants is maximal when they are located in the same phase where lipid oxidation occurs [1].

Unlike BHT, which dissolves passively in fat, SIL-O appears to become an integral part of the lipid microstructure, which is in agreement with recent literature showing that esterified derivatives of polyphenols exhibit a more uniform distribution and increased stability in complex lipid systems [11].

The second differences are visible in the range of 1750–1700 cm^{-1} characteristics for – C=O vibrations (esters). The intense band around 1743–1745 cm^{-1}, associated with stretching vibrations of ester C=O groups in triglycerides, is present in all samples.

In the Control sample, this band has a symmetrical shape and a constant intensity, characteristic of a relatively stable lipid system.

The 200 ppm BHT sample does not show significant changes in the position of the C=O band, confirming that BHT does not contain ester carbonyl groups and does not directly influence the structure of the esters in butter. This observation is in agreement with the literature data showing that BHT does not modify FTIR spectrum of lipids in the carbonyl region, even though it improves oxidative stability.

In contrast, samples S1–S3 show a slight increase in intensity and a discrete broadening of the C=O band, more pronounced in S2 and S3. This effect is attributed to the overlap of the C=O ester vibrations of triglycerides with those of the ester group in silibinin-oleate. Literature confirms that SIL-O shows a characteristic C=O ester band in the same spectral range, and the increase in intensity of this region is an indicator of its efficient incorporation into the lipid phase [11].

The intensification and broadening of the C=O band (≈1744 cm^{-1}) in samples fortified with SIL-O confirms the additional presence of ester groups originating from silibinin-oleate. This observation has important functional implications, since esterification of silibinin with fatty acids increases the bioavailability and chemical stability of the compound, without compromising the antioxidant activity.

Thus, FTIR data suggest that SIL-O is not only present in butter, but also retains its molecular functionality, an essential aspect for classifying the product as a functional food.

The range 1650–1600 cm^{-1} corresponds to aromatic structures and C=C bonds. In the Control sample, this region is poorly represented, reflecting the low content of natural aromatic compounds in butter.

For BHT 200 ppm, a slight increase in absorption occurs around 1605–1610 cm^{-1}, corresponding to the C=C vibrations of the aromatic nucleus of BHT. The relatively low intensity is explained by the low concentration and by the fact that BHT is not covalently bound to the lipid matrix. In samples S1–S3, this region becomes more evident, especially in S2 and S3, where a more clearly defined absorption between 1600–1630 cm^{-1} is observed. This is attributed to the flavonolignan structure of silibinin, which contains several conjugated aromatic nuclei. According to recent FTIR studies, the intensification of aromatic bands is a direct marker of the presence of silibinin or its lipophilic derivatives in food systems [9].

The enhancement of the bands in the 1600–1630 cm^{-1} region, associated with conjugated aromatic structures, indicates the maintenance of the active flavonolignan core of silibinin. Literature demonstrates that the aromatic cores and phenolic groups are responsible for the free radical scavenging capacity and pro-oxidant metal ion chelation, essential mechanisms for the protection of dietary lipids [11].

The spectral region 1200–1000 cm^{-1} corresponds to – C–O and C–O–C vibrations. This region is particularly relevant for sample differentiation. In the Control sample, the bands are predominantly associated with C–O bonds in glycerol and fatty acid esters.

The 200 ppm BHT sample shows only minor changes, which again confirms the lack of significant structural interactions between BHT and

the lipid matrix.
In contrast, samples S1, S2 and S3 show a progressive intensification of the bands in this range, correlated with the level of SIL-O. This evolution is associated with the hydroxyl and ether groups of silibinin, even if partially esterified. The literature shows that these bands are sensitive to the concentration of phenolic compounds and can be used as a spectroscopic indicator of fortification with natural antioxidants.

Conclusions

Compared to BHT, which acts predominantly as a radical antioxidant without significantly altering the spectroscopic structure of the lipids, SIL-O induces detectable and concentration-dependent FTIR changes, confirming its structural integration into the butter fat matrix. This observation is supported by recent studies on lipophilic derivatives of silibinin, which report superior compatibility with lipid systems, increased stability and a more uniform molecular distribution compared to synthetic antioxidants.
Compared to butter treated with 200 ppm BHT, the samples with SIL-O show distinct spectral signatures, especially in the C=O, C–O and aromatic regions. This difference reflects an important conceptual distinction. BHT acts predominantly as a technological antioxidant, with an oxidation retarding role, without additional biological value for the consumer. SIL-O, on the other hand, combines oxidative protection of the lipid matrix with biologically active potential (antioxidant, hepatoprotective, anti-inflammatory).
This difference is directly reflected in the FTIR spectra, where SIL-O induces detectable structural changes, while BHT does not produce relevant spectroscopic variations.
The progressive differences between S1, S2 and S3 indicate that FTIR can be used as a rapid method to confirm fortification and discriminate the level of SIL-O in butter, which represents an important methodological advantage in the development of functional dairy products.

Conflicts of interest / Competing interests: No conflict of interest

Ethics approval: No ethic situation.

Availability of data and material: The data have been reviewed depending on the literature.

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