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Fatty-acids Composition of Butter Fortified with Silibinin-Oleate

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Abstract
Butter is a complex lipid matrix prone to oxidative degradation due to the presence of unsaturated fatty acids. This study investigated the effect of silibinin oleate (SIL-O), a lipophilic derivative of the flavonoid silibinin, on the fatty acid profile and lipid-related indices of cow's milk butter, in comparison with a conventional synthetic antioxidant, butylated hydroxytoluene (BHT). Sweet cream butter (82% fat, unsalted) was supplemented with SIL-O at 100, 200, and 300 ppm, while control and BHT-treated samples were prepared under identical conditions. Fatty acid composition was determined by GC-FAME analysis, and lipid indices including saturated (SFA), unsaturated (UFA), and polyunsaturated fatty acids (PUFA), UFA/SFA and PUFA/SFA ratios, and the unsaturation index (UI) were calculated. Butter fat was dominated by SFA (≈65–66%), mainly palmitic, myristic, and stearic acids. SIL-O supplementation resulted in higher UFA and PUFA proportions, increased UFA/SFA and PUFA/SFA ratios, and elevated UI values compared with control and BHT samples. These results indicate a moderate increase in lipid unsaturation while maintaining the characteristic fatty acid profile of butter, supporting the potential of silibinin oleate as a natural antioxidant in dairy fat systems.
Keywords: saturated (SFA), unsaturated (UFA), polyunsaturated fatty acids (PUFA), UFA/SFA, PUFA/SFA ratios, unsaturation index (UI)

1. Introduction

Butter is a semi-solid lipid system obtained through the concentration of milk fat, in which triacylglycerols represent the dominant fraction, accompanied by minor constituents such as phospholipids, cholesterol, pigments, and tocopherols. From a compositional perspective, milk fat is characterized by a high level of complexity, with approximately 400 fatty acids identified; however, its profile is primarily governed by a limited number of major components. In general, butter fat is predominantly composed of saturated fatty acids (approximately 70–75%), alongside significant contributions from mono- and polyunsaturated fatty acids [1].

In cow's milk butter, palmitic acid (C16:0) is typically the most abundant saturated fatty acid, accounting for approximately 25–30% of total fatty acids, followed by myristic (C14:0) and stearic (C18:0), each generally present at levels around 10–13%, depending on production conditions. Among unsaturated fatty acids, oleic acid (C18:1 cis-9) is

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usually the predominant component, while linoleic (C18:2) and α-linolenic acids (C18:3) occur in lower proportions but are of considerable nutritional and oxidative importance. Data from commercial butter samples indicate substantial variability in fatty acid composition, particularly for palmitic and oleic acids, reflecting the strong influence of environmental and production factors [2–4].

This variability is primarily attributed to biological and technological determinants, including animal species and breed, stage of lactation, seasonal variation, and especially feeding regime (e.g., pasture-based versus concentrate-rich diets), as well as processing parameters such as cream separation, pasteurization, maturation, moisture content, and salting. Recent studies have shown that butter derived from grazing systems tends to exhibit a more unsaturated lipid profile, with increased levels of beneficial components such as conjugated linoleic acid (CLA) and omega-3 fatty acids (e.g., α-linolenic acid), although these effects depend on grazing intensity and lactation stage [5].

The technological functionality of butter, including its firmness, plasticity, spreadability, and melting behavior, is largely governed by the triacylglycerol composition and the balance between saturated and unsaturated fatty acids. Saturated fatty acids contribute to higher melting points and increased crystallinity, whereas unsaturated fatty acids enhance fluidity.

In this context, palmitic acid (C16:0), due to its relatively high proportion, plays a key role in determining the physico-rheological properties of butter fat [6].

From an application perspective, relatively small variations in the C16:0/C18:1 ratio, as well as in the proportion of short- and medium-chain fatty acids, can significantly influence key technological properties of butter, including hardness at refrigeration temperatures, melting behavior (critical for mouthfeel), and structural stability in baked products [7].

Although butter contains a high proportion of saturated fatty acids, which are generally more resistant to oxidation, the presence of unsaturated fatty acids, cholesterol, and photosensitizing compounds renders it susceptible to lipid oxidation through both auto-oxidative and photo-oxidative mechanisms.

Oxidation processes initially lead to the formation of hydroperoxides as primary products, followed by the generation of secondary compounds such as aldehydes, ketones, and other volatile molecules responsible for rancidity and sensory deterioration. Consequently, recent research has increasingly focused on the use of natural antioxidants to delay the progression of oxidation and improve the oxidative stability of butter during storage [8].

Photo-oxidation represents a particularly critical factor, especially when butter is exposed to light through transparent packaging or under retail display conditions. Both classical and recent studies have documented the susceptibility of butter lipids, including cholesterol, to light-induced oxidation, as well as strategies to mitigate these effects through the use of protective pigments, functional ingredients, and optimized packaging systems [9,10].

Temperature is another key factor influencing oxidation kinetics, typically following Arrhenius-type behavior, while prolonged storage promotes the accumulation of secondary oxidation products that significantly impact flavor quality. Recent studies on butter oil and anhydrous milk fat have also explored stabilization strategies under industrial conditions, including the controlled addition of antioxidants such as α-tocopherol to enhance oxidative resistance during processing and storage [11].

From a nutritional perspective, an increased proportion of polyunsaturated fatty acids (PUFA), such as α-linolenic acid (ALA, n-3) associated with pasture-based feeding systems, may improve the lipid profile of butter. However, this shift may also enhance oxidative susceptibility in the absence of adequate antioxidant protection and packaging solutions [12].

At the same time, naturally occurring antioxidants, including tocopherols and carotenoids, contribute to the intrinsic oxidative stability of butter. Several studies on commercial butter have reported simultaneous evaluation of compounds such as α-tocopherol and β-carotene in relation to product quality and shelf-life [13].

In this context, the present study aims to investigate the role of a natural antioxidant obtained through green synthesis, silibinin oleate (SIL-O), as a protective agent against lipid oxidation in the butter fat matrix. The oxidative stability was evaluated through the analysis of fatty acid profiles in samples treated with different concentrations of SIL-O, in comparison with the synthetic antioxidant butylated hydroxytoluene (BHT).

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 formulations. Silibinin oleate (SIL-O) was synthesized and purified according to previously reported procedures based on the esterification of flavonoids with fatty acids [14]. Butylated hydroxytoluene (BHT, ≥99% purity) was purchased from a certified chemical supplier and used as a synthetic antioxidant reference. All solvents and reagents employed in the study were of analytical or HPLC grade.

2.2. Preparation of butter samples

Butter samples were melted in a water bath at 40–45 °C to obtain a homogeneous lipid phase while minimizing thermal degradation. SIL-O was first dissolved in a small portion of melted butter to ensure complete dispersion, and subsequently incorporated into the bulk butter to achieve final concentrations of 100 ppm (S1), 200 ppm (S2), and 300 ppm (S3), expressed on a fat basis.

A reference sample containing 200 ppm BHT was prepared using the same procedure. Control samples were processed under identical conditions, without the addition of antioxidants.

2.3. Fatty acids analysis

The fatty acid composition was determined following a transesterification procedure, with subsequent analysis of fatty acid methyl esters (FAMEs) by gas chromatography (GC), as previously described [14].

Fatty acid–based indices were calculated from the GC-FAME data by grouping fatty acids into saturated (SFA, Cx:0), unsaturated (UFA, Cx:y, $y \geq 1$), and polyunsaturated (PUFA, $y \geq 2$) fractions. The ratios UFA/SFA and PUFA/SFA were used to characterize the degree of lipid unsaturation. The unsaturation index (UI) was calculated as the sum of each unsaturated fatty acid multiplied by its corresponding number of double bonds [14].

2.4. *Statistical analysis*

All analyses were performed in triplicate, and the results are expressed as mean values ± standard deviation (SD). Statistical differences between samples were evaluated using a two-sample t-test assuming equal variances, performed in Microsoft Excel 365. Differences were considered statistically significant at $p < 0.05$.

3. Results and discussions

The data set suggests a stabilizing effect of SIL-O on the unsaturated fatty acid fractions, without inducing systematic dose-dependent changes in the main saturated fatty acids. This behavior is consistent with the expected function of an antioxidant, which primarily limits autoxidation reactions rather than altering the intrinsic lipid composition. From a chemical perspective, SIL-O represents a lipophilic esterified derivative of silibinin (a flavonolignan), designed to enhance its affinity for lipid matrices and, consequently, its effectiveness in fat-rich systems [15].

The results presented in Table 1 indicate that short- and medium-chain saturated fatty acids (e.g., C8:0, C10:0, C12:0) exhibit only minor variations between the control and SIL-O-treated samples (100–300 ppm), without a consistent monotonic trend. This supports the interpretation that SIL-O does not modify the primary fatty acid composition of butter fat. Any potential effect is more likely related to the limitation of subsequent oxidative transformations, which are not directly quantified in the present analysis.

This observation is particularly relevant given that the fatty acid profile of butter is dominated by saturated fatty acids—especially palmitic, stearic, and myristic acids—as well as monounsaturated fatty acids. Significant variations in these fractions are typically associated with factors such as animal diet, seasonal variation, or raw material origin, rather than with the addition of antioxidants [16].

Within the MUFA fraction, oleic acid (C18:1 cis-9) remained relatively stable across all samples, while palmitoleic acid (C16:1) showed a more pronounced decrease in the SIL-3 (300 ppm) variant compared to SIL-1 and SIL-2. This variation is more likely attributable to analytical variability or matrix-related effects (e.g., heterogeneous distribution of the antioxidant within the lipid phase) rather than a direct biochemical or metabolic effect. In general, antioxidants in food systems exert their action by interrupting radical chain reactions (through hydrogen donation, radical scavenging, or metal chelation), and their efficiency depends strongly on the microstructure of the lipid matrix and storage conditions.

The polyunsaturated fatty acid (PUFA) fraction, which is the most susceptible to oxidative degradation, represents the key target for antioxidant action. In full-fat dairy systems, differences between antioxidant treatments are typically more evident in oxidation indicators (e.g., peroxide value, TBARS, volatile compounds) [16], although indirect effects may also be observed through the preservation of

unsaturated fatty acids over time. In Table 1, linoleic acid (C18:2) values in SIL-O-treated samples remain within a narrow range (~3.15–3.21%), while other PUFA components (e.g., ALA, GLA, ARA, DHA) show only minor variations between samples. These findings may be interpreted as an overall stabilization of PUFA in the presence of SIL-O, without a clear dose-dependent enhancement across the tested concentration range (100–300 ppm).

Recent literature highlights a growing interest in natural or naturally derived antioxidants for butter stabilization, aiming to reduce reliance on synthetic additives while preserving sensory quality. For instance, Çakmakçı et al. (2023) investigated the incorporation of polyphenol-rich green tea powder in butter and compared its antioxidant and sensory effects with those of conventional antioxidants such as BHT [17]. Similarly, Martoglu et al. (2024) evaluated oxidation kinetics in lactic butter supplemented with essential oils, demonstrating the potential of natural compounds to enhance oxidative stability [18]. Given its lipophilic nature, SIL-O exhibits strong mechanistic potential to act effectively within the lipid phase of butter. However, the tested concentration range (100–300 ppm) appears to approach a plateau effect, suggesting that antioxidant efficiency in such systems is not strictly linear. This observation is consistent with current understanding that antioxidant performance in real food matrices depends on multiple factors, including distribution within the lipid phase, the presence of pro-oxidants, and the pathways of oxidative degradation [16].

The analysis of the fatty acid profile indicates that cow's butter is predominantly composed of saturated fatty acids (SFA), accounting for approximately 65–66% of the total quantified fatty acids, irrespective of the treatment applied (Control, BHT, or SIL-O). This distribution is characteristic of dairy fat and is consistent with recent literature reports, which indicate typical SFA contents ranging from 60% to 75% in commercial butter samples [4,12].

Within the SFA fraction, palmitic acid (C16:0) represents the dominant component, followed by myristic (C14:0) and stearic (C18:0) acids. These fatty acids are widely recognized as key contributors to the physicochemical properties of butter, including consistency, melting behavior, and plasticity. The values obtained in the present study fall within the ranges reported by Grille et al., who identified palmitic acid as the major fatty acid (~22–30%), with myristic and stearic acids typically present at levels of approximately 9–13% [3].

Table 1. Fatty acids composition of butter samples with SIL-O

Fatty acids	Commercial name	RT	Control	BHT	SIL-1	SIL-2	SIL-3
Butiric acid (C4:0)		2.380	3.560±0.05^{a}	3.544±0.02^{a}	3.223±0.01^{b}	3.229±0.05^{b}	3.230±0.04^{b}
Caproic acid (C6:0)		2.630	2.240±0.01^{c}	2.356±0.08^{b}	2.345±0.10^{b}	2.387±0.10^{a}	2.390±0.05^{a}
Octanoic acid (C8:0)	**Caprylic acid**	3.255	1.038±0.04^{b}	1.145±0.02^{a}	0.966±0.05^{b}	0.980±0.02^{b}	0.988±0.01^{b}
Decanoic acid (C10:0)	**Capric acid**	4.806	2.765±0.04^{a}	2.601±0.08^{c}	2.603±0.05^{c}	2.579±0.05^{c}	2.612±0.02^{b}
Undecanoic acid (C11:0)	**Undecylic acid**	6.061	0.071±0.01^{a}	0.067±0.01^{a}	0.067±0.01^{a}	0.066±0.01^{a}	0.068±0.01^{a}
Dodecanoic acid (C12:0)	**Lauric acid**	7.582	3.516±0.02^{a}	3.357±0.05^{b}	3.327±0.02^{b}	3.341±0.04^{b}	3.371±0.02^{b}
Tridecanoic acid (C13:0)	-	9.230	0.124±0.02^{a}	0.120±0.01^{a}	0.119±0.02^{a}	0.120±0.01^{a}	0.121±0.02^{a}
Myristic acid (C14:0)	**Myristic acid**	**10.940**	**11.120±0.4^{a}**	**10.817±0.50^{b}**	**10.701±0.20^{b}**	**10.869±0.40^{b}**	**10.873±0.50^{b}**
cis-9 Myristoleic (C14:1)	**Myristoleic acid**	11.584	0.898±0.02^{a}	0.856±0.02^{b}	0.844±0.01^{b}	0.852±0.01^{b}	0.859±0.05^{b}
Pentadecanoic acid (C15:0)	**Pentadecylic acid**	12.605	1.259±0.01^{a}	1.229±0.01^{a}	1.229±0.01^{a}	1.240±0.02^{a}	1.239±0.02^{a}
cis-10	-	13.250	0.014±0.005^{a}	0.014±0.005^{a}	0.013±0.005^{a}	0.014±0.008^{a}	0.014±0.008^{a}

Pentadecanoic (C15:1)							
Palmitic acid (C16:0)	**Palmitic acid**	**14.240**	**29.071±1.20^{a}**	**28.824±0.50^{a}**	**28.732±1.00^{a}**	**29.153±1.20^{a}**	**28.921±1.00^{a}**
cis-9 Palmitoleic (C16:1)	**Palmitoleic acid**	14.670	0.312±0.02^{a}	0.305±0.01^{b}	0.299±0.008^{b}	0.294±0.005^{b}	0.197±0.005^{c}
Margaric acid (C17:0)	-	15.760	0.630±0.05^{b}	0.658±0.05^{a}	0.669±0.02^{a}	0.635±0.01^{b}	0.630±0.01^{b}
cis-10 Heptadecenoic (C17:1)	-	16.204	0.283±0.005^{b}	0.301±0.008^{a}	0.278±0.005^{b}	0.275±0.008^{b}	0.278±0.005^{b}
Stearic acid (C18:0)	**Stearic acid**	**17.502**	**10.785±1.00^{c}**	**10.845±0.08^{b}**	**10.884±0.08^{b}**	**11.045±0.10^{a}**	**10.837±0.50^{b}**
cis-9 Oleic acid (C18:1)	**Oleic acid**	**17.840**	**24.653±0.80^{a}**	**24.806±1.2^{a}**	**24.576±1.00^{a}**	**24.748±1.00^{a}**	**24.861±0.80^{a}**
trans-9,12 Linoleic	**Linolelaidic acid**	17.980	0.084±0.005^{a}	0.084±0.005^{a}	0.083±0.008^{a}	0.083±0.002^{a}	0.085±0.005^{a}
cis-9,12 Linoleic acid (C18:2)	**Linoleic acid**	18.435	0.024±0.004^{a}	0.025±0.002	3.206±0.05^{a}	3.147±0.05^{a}	3.154±0.04^{a}
γ-Linolenic (6,9,12 C18:3)	**Gamma-linolenic acid (GLA)**	18.775	0.111±0.02^{b}	0.121±0.01^{a}	0.109±0.02^{b}	0.115±0.02^{b}	0.111±0.02^{b}
α-Linolenic (9,12,15 C18:3)	**Alpha-linolenic acid (ALA)**	19.235	0.525±0.04^{b}	0.515±0.05^{c}	0.539±0.04^{a}	0.511±0.05^{c}	0.513±0.03^{c}
Arachidic acid (C20:0)	**Arachidic acid**	20.027	0.025±0.005^{b}	0.024±0.002^{b}	0.033±0.001^{a}	0.023±0.001^{b}	0.024±0.001^{b}
cis-11 Eicosenoic (C20:1)	**Gondoic acid**	20.383	0.062±0.001^{a}	0.068±0.001^{a}	0.065±0.001^{a}	0.063±0.001^{a}	0.065±0.001^{a}
cis-11,14 Eicosadienoic (C20:2)	**Eicosadienoic acid**	20.8	0.020±0.001^{a}	0.022±0.001^{a}	0.018±0.002^{a}	0.017±0.002^{a}	0.022±0.002^{a}
cis-8,11,14 Eicosatrienoic (C20:3)	**Dihomo-gamma-linolenic acid (DGLA)**	21.015	0.066±0.002^{a}	0.066±0.002^{a}	0.063±0.002^{a}	0.058±0.004^{a}	0.065±0.004^{a}
Heneicosanoic acid (C21:0)	-	21.290	0.010±0.001^{a}	0.007±0.001^{a}	0.005±0.001^{b}	0.004±0.001^{b}	0.010±0.002^{a}
cis-5,8,11,14 Eicosatetraenoic (C20:4)	**Arachidonic acid (ARA)**	21.713	0.176±0.004^{a}	0.171±0.002^{a}	0.168±0.002^{b}	0.166±0.002^{b}	0.174±0.004^{b}
cis-11,14,17 Eicosatrienoic (C20:3 ω-3)	**Eicosatrienoic acid (ETA)**	21.921	0.022±0.001^{a}	0.019±0.001^{s}	0.013±0.002^{b}	0.010±0.002^{b}	0.024±0.002^{s}
Behenic acid (C22:0)	**Behenic acid**	22.554	0.066±0.001^{b}	0.061±0.002^{b}	0.108±0.002^{a}	0.050±0.004	0.069±0.004^{b}
cis-13 Erucic acid (C22:1)	**Erucic acid**	22.916	0.033±0.001^{a}	0.023±0.002^{b}	0.038±0.001^{a}	0.019±0.001^{b}	0.034±0.002^{a}
Tricosanoic acid (C23:0)	-	24.071	0.030±0.001^{a}	0.031±0.002^{a}	0.031±0.002^{a}	0.031±0.002^{a}	0.032±0.002^{a}
cis-4,7,10,13,16,19 (C22:6)	**Docosahexaenoic acid (DHA)**	25.8	0.020±0.002^{a}	0.023±0.001^{a}	0.023±0.001^{a}	0.009±0.001^{b}	0.019±0.001^{a}

Values are expressed as mean ± standard deviation (n=3). The statistical analysis performed include different superscript letters (e.g., a, b, c, d) within the same row indicate significant differences between samples according to Student's t-test ($p < 0.05$).

The fraction of unsaturated fatty acids (UFA = MUFA + PUFA) represents approximately 27–30%, with a clear increase in samples treated with silibinin oleate (SIL) compared to Control and BHT. This increase is directly reflected in the UFA/SFA ratio, which goes from values of ~0.41–0.42 (Control, BHT) to ~0.46 in SIL samples.

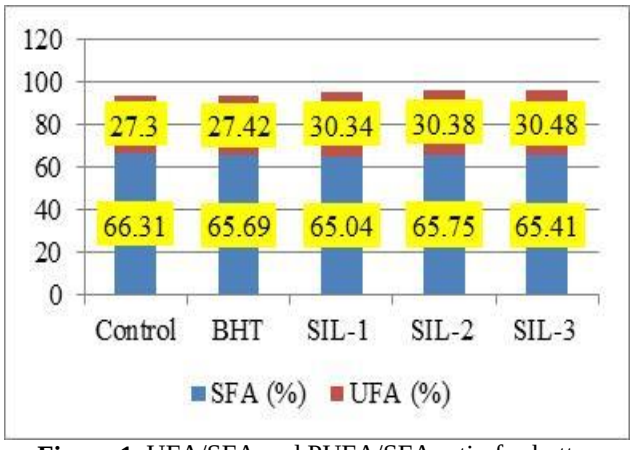

Figure 1. UFA/SFA and PUFA/SFA ratio for butter samples with SIL-O

Recent studies report UFA/SFA ratio values in butter typically ranging from 0.33 to 0.48, depending on factors such as animal diet, seasonal variation, and production system [4,12]. In this context, the values obtained for the SIL-O-treated samples fall within the upper range of those reported in the literature, suggesting a relatively higher degree of lipid unsaturation.

The increase in the UFA fraction observed in the SIL-O samples is primarily driven by the contribution of polyunsaturated fatty acids (PUFA), as reflected by the higher PUFA/SFA ratios (approximately 0.063–0.065 in SIL-O samples compared to approximately 0.016 in the Control and BHT samples). These values are comparable to those reported for butter produced under conditions associated with enhanced PUFA content, such as pasture-based feeding systems or specific seasonal influences [12].

From a nutritional perspective, a higher proportion of unsaturated (UFA) and polyunsaturated fatty acids (PUFA) is generally considered beneficial. However, from a technological and oxidative standpoint, an increased degree of unsaturation is associated with greater susceptibility to lipid oxidation. This aspect is particularly relevant in the context of the present study, as it underscores the necessity of incorporating effective antioxidants, such as silibinin oleate (SIL-O), to stabilize a more unsaturated lipid matrix.

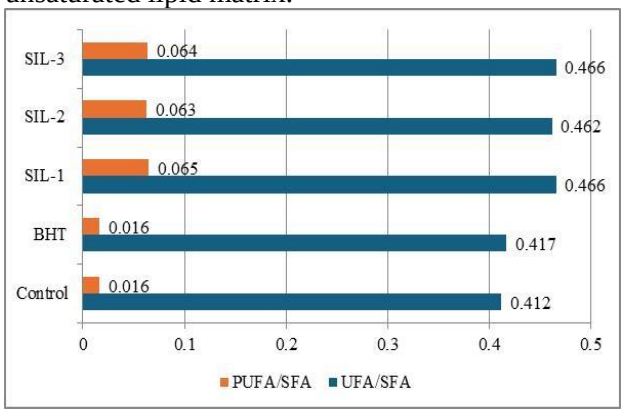

Figure 2. PUFA/SFA ratio for butter samples with SIL-O

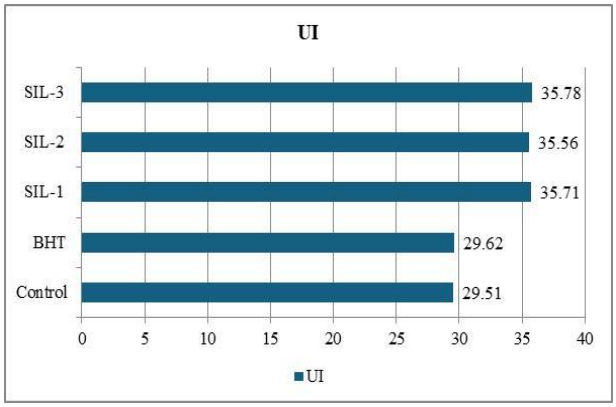

Figure 3. UI/DBI ratio for butter samples with SIL-O

The unsaturation index (UI) calculated for the analyzed samples further supports this trend. Control and BHT-treated samples exhibited UI values of approximately 29–30, consistent with those typically reported for conventional butter. In contrast, SIL-O-treated samples showed higher UI values (~35–36), indicating a greater density of double bonds and, consequently, an increased potential for oxidative reactivity.

Conclusions

The results confirm that the analyzed butter exhibits a characteristic milk fat composition, dominated by saturated fatty acids (SFA) while maintaining a significant proportion of unsaturated fatty acids (UFA). Overall, supplementation with SIL-O at concentrations of 100–300 ppm preserved a comparable fatty acid profile across samples and did not induce systematic changes in the SFA or MUFA fractions. This finding supports the hypothesis that SIL-O primarily exerts a protective and stabilizing effect, particularly with respect to the more oxidation-prone unsaturated components.

Furthermore, the absence of a clear dose–response relationship among the SIL-1, SIL-2, and SIL-3 variants suggests that, under the experimental conditions applied, concentrations in the range of 100–200 ppm may be sufficient to achieve the desired stabilizing effect. Increasing the concentration to 300 ppm does not appear to provide a significant additional benefit in terms of fatty acid composition.

Conflicts of interest / Competing interests: No conflict of interest

Ethics approval: No ethic situation.

Availability of data and material: All data generated or analyzed during this study are included in this published article.

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