EXPLORING FAT REPLACEMENT IN CHEESE ANALOGUES *VIA* ^{1}H- AND ^{13}C-NMR SPECTROSCOPY, LIPID NUTRITIONAL DESCRIPTORS, RHEOLOGICAL BEHAVIOR AND COLOR

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This study investigates the development of cheese analogues obtained by partial substitution of milk fat with sunflower and rapeseed oils at different levels (25% and 50%). The samples were evaluated using ^{1}H- and ^{13}C-NMR spectroscopy, lipid nutraceutical indices, rheological and colorimetric analyses. Substitution with vegetable oils significantly improved the lipid profile, lowering atherogenic and thrombogenic indices while increasing the PUFA/SFA ratio, with rapeseed oil showing the best results. Rheological data indicated enhanced elasticity, while color parameters remained within acceptable limits. These findings highlight vegetable oil-based cheese analogues as healthier alternatives to conventional cheese with favorable nutritional and physicochemical properties.

Keywords: cheese analogues; milk fat; nutritional scores; NMR spectroscopy; rapeseed oil; sunflower oil.

1. Introduction

Owing to its complex flavors and varied textures, cheese holds a distinguished position in culinary traditions across the globe. However, its consumption can be associated with certain health concerns. One potential issue is the presence of pathogenic microorganisms, particularly in unpasteurized or

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improperly handled cheese, which can lead to foodborne illnesses such as listeriosis or salmonellosis [1]. Additionally, cheese is often high in saturated fats and sodium, which may contribute to increased cardiovascular risk if consumed in excess. Therefore, it is important to explore and develop healthier alternatives to conventional cheese, such as cheese analogs in which dairy fat is partially replaced with plant-based oils.

Cheese analogues are products intended to replace traditional cheeses by replacing milk fats and/or proteins with vegetable alternatives [2]. The market for these types of products is constantly growing because they are easy to obtain and involve lower production costs than traditional cheeses [3]. Consequently, cheese analogues can serve as cheese-like food products with similar properties mimicking the unique flavor of cheese, however with lower costs [4].

Cheese analogues are obtained by partially substituting milk fat with affordable vegetable oils rich in polyunsaturated fatty acids. The latter are used to improve the fatty acid profile of dairy products [5]. Enrichment of dairy products with n-3/n-6 fatty acids has, until recently, been achieved mainly by including oilseeds and vegetable oils in animal feed [6,7]. Another possibility that has been tried is the partial or total replacement of milk fat with oils rich in polyunsaturated fatty acids [8]. Although the substitution of milk fats with vegetable oils improves the fatty acid profile of the final product [9], this process comes with important challenges. One of these is reproducing the functionality of classic cheese, since these milk modifications can affect the physical properties and stability of cheese analogues [10]. Therefore, cheese analogue formulations tend to avoid drastic changes to the classic technological processes of obtaining cheese after replacing milk fat with vegetable oil [11].

The fatty acids composition of edible fats and oils has an impact on health [12]. In this regard, fats rich in saturated fatty acids (*e.g*. dairy fats [13]) stimulate cholesterol synthesis (hypercholesterolemic effect) in the liver and its deposition on the walls of blood vessels in the form of atheroma, which over time may lead to cardiovascular diseases. On the other hand, vegetable oils, with a high content of polyunsaturated fatty acids, inhibit cholesterol synthesis in the liver, while also contributing to the solubilization of cholesterol sediments deposited on the walls of blood vessels, thus exhibiting a hypocholesterolemic effect [14]. Therefore, in the case of adults, it is recommended to reduce the proportion of saturated fats in the diet in order to prevent the risk of cardiovascular diseases [15].

Due to their high saturated fatty acid content, dairy fats lead to increased LDL levels [16,17]. For this reason, attempts are being made to replace animal fat in dairy products with vegetable oils to obtain dairy analogues. These analogues represent healthier alternatives that retain most of the characteristics of classic cheeses, while also featuring a significant amount of ω–6 and ω–9 fatty acids that contribute to maintaining an optimal level of LDL cholesterol [2].

In scientific literature, there are studies addressing cheese analogues with modified fat compositions achieved through partial substitution with vegetable oils; however, relatively few investigations have focused on formulations containing high proportions of sunflower or rapeseed oil for white cheese analogues. Instead, research has predominantly targeted the production of analogues of other cheese types, such as Cheddar [18], Gouda [19], Kashar [20], and Oaxaca types [21], among others.

On the other hand, some studies do not focus on the characterization of cheese analogues *per se*, but rather on the effects of their consumption on cholesterol levels. For example, a study conducted in Finland [22] demonstrated that consumption of cheese partially substituted with rapeseed oil significantly reduced total and LDL cholesterol compared to control cheese. However, this study did not extensively address texture, color, or fat characterization through health descriptors such as the atherogenic or thrombogenic indices.

Conversely, other investigations comprehensively characterize cheese analogues from rheological, textural, and sensory perspectives but do not evaluate the lipid profile using health indices. For instance, the impact of partially replacing milk fat with sunflower oil on the microstructure, rheological properties, and texture profile of cheese analogues—where milk fat was substituted at various levels (10%, 20%, 30%, 40%)—suggests that partial substitution significantly influences the textural and rheological characteristics of cheese analogues depending on the substitution level, without reference to health impacts [23].

Therefore, the present article aims to fill a gap in the literature by evaluating cheese analogues obtained through partial substitution with vegetable oils (sunflower and rapeseed), both in terms of rheological and colorimetric parameters and, importantly, through assessment of the lipid profile using specific nutraceutical descriptors such as the atherogenic and thrombogenic indices. Moreover, the lipid fraction is characterized using ^{1}H- and ^{13}C-NMR spectroscopy, highlighting spectral differences between control and cheese analogues.

2. Materials and methods

2.1 *Chemicals*

Semi-skimmed cow milk (1.5g/100g fat content), sunflower oil and rapeseed oil were purchased from a local hypermarket. Anhydrous $CaCl_2$ and $MgSO_4$ were purchased from Merck. Sulfuric acid was purchased from Sigma-Aldrich. Deuterated chloroform ($CDCl_3$) was purchased from Supelco. Double distilled water (dd H_2O) was used in the experiments.

2.2 *Experimental design*

Three cheese analogues with various ratios of vegetable oil – 25% sunflower oil (CA-25%-SF), 50% sunflower oil (CA-50%-SF), 50% rapeseed oil (CA-50%-

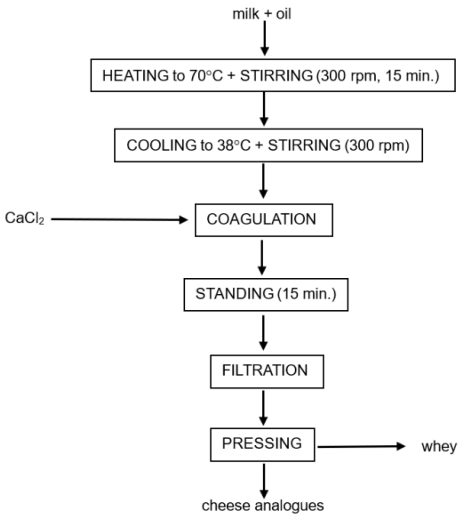
R), respectively – and a Control sample (obtained from 100% milk) were prepared according to the experimental design presented in *Table 1*.

Table 1

Experimental design for preparing the cheese analogues

Sample	Ingredients				Curd [g]	Whey [g]
	Skimmed milk (1.5% fat) [mL]	Oil		$CaCl_2$[mL]		
		Sunflower [g]	Rapeseed [g]			
Control	200	-	-	15	34	167
CA-50%-SF	200	1	-	15	21	184
CA-25%-SF	200	3	-	15	22	180
CA-50%-R	200	-	3	15	22	181

Milk was initially heated to 70°C with oil, under magnetic stirring (300 rpm) for homogenization, then allowed to cool to 38°C under continuous stirring, at which point aqueous $CaCl_2$ solution (1M) was added as a clotting agent. The mixture was then left to stand for 15 minutes, to allow the formation of the coagulum. The whey was removed by filtration through a sieve (truncated cone shape) covered with triple-layered sterile tissue. Light pressure was applied at the end of the filtration so that the curd takes on the desired truncated cone shape. The steps for obtaining cheese analogues are shown in *Scheme 1*. Samples appearance is shown in *Fig. 1*.



Scheme 1: Unit operations for obtaining cheese analogues

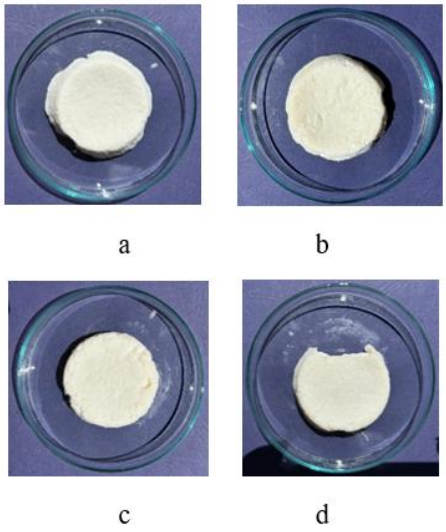


Fig. 1: Visual appearance of the samples: a – Control; b – CA-50%-SF; c – CA-25%-SF; d – CA-50%-R

2.3 Fat extraction

Fat content was determined using the Schmidt-Bonzynsky-Ratzaloff method [24]. Briefly, 1.5 g of the sample was digested with 10 mL of HCl aqueous

sol. (1M) on a water bath at 90 °C for 40 minutes. After cooling to room temperature, lipids were extracted with three portions of 15 mL diethyl ether/petroleum ether mixture (1:1). The combined etheric phases were evaporated, and the fat content was determined gravimetrically and expressed as g fat/100 g product.

2.4 NMR characterization

^{1}H- and ^{13}C-NMR experiments were recorded on a Bruker Advance III HD 600 MHz spectrometer (Bruker, Rheinstetten, Germany), corresponding to the resonance frequency of 600.12 MHz for the ^{1}H nucleus and 150.90 MHz for the ^{13}C nucleus, equipped with a direct detection four nuclei probehead and field gradients on the *z* axis. Samples were analyzed in 5 mm NMR tubes (Wilmad 507). The NMR samples were prepared by dissolving 0.5 mL of oil in 0.5 mL $CDCl_3$. Chemical shifts are reported in ppm, relative to the $CDCl_3$ signal as internal standard (77.16 ppm). Typical parameters for semi-quantitative ^{13}C-NMR spectra were: 30° pulse, 1s acquisition time, 30s relaxation time, 32 KHz spectral window, 64 scans, 64 K data points, using an inverse-gated pulse sequence to minimize the NOE effects as described in the literature [25]. After recording, phase and baseline corrections, calibration and signal integration were done using the *TopSpin 3.5PL6* software package.

2.5 Rheological analysis

The rheological properties of the cheese samples were evaluated using a Kinexus Pro oscillatory rheometer (Malvern Instruments, Belgium) with flat upper and lower geometry, equipped with a Peltier element for precise temperature control. Samples were cut as disks of 1 cm diameter and 1 mm thick, placed on the lower plate of the rheometer, and then the upper plate was lowered over the sample and covered by the protective covers. Frequency sweep measurements were recorded between 0.10–10 Hz at 25 ºC to determine the storage modulus (G') and the loss modulus (G'') at a constant shear stress of 1 Pa.

2.6 Color analysis

Color parameters were measured in the CIE L*a*b* space using a Jasco 670 spectrometer in the UV-Vis domain, from 300-800 nm, provided with a diffuse reflectance integrating sphere. The objective analysis of the sample colour has been performed with the instrument's software. The calibration of the instrument was done before measurements and then colour parameters L* [lightness 0 (dark)-100 (light)], a* [green (−)-red (+)], and b* [blue (−)-yellow (+)] were automatically recorded. Chroma (C^*), hue (H^0), and colour difference (ΔE) were further

calculated [26] using the following equations, taking the Control sample as a reference.

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$H^0 = \text{arctg}(\frac{b^*}{a^*}) \quad (2)$$

$$\Delta E = [(L_0{}^* - L^*)^2 + (a_0{}^* - a^*)^2 + (b_0{}^* - b^*)^2]^{1/2} \quad (3)$$

where L_0*, a_0* and b_0* represent the colour parameters of the Control. All colour measurements were performed in triplicate across all the experiments. Data are reported as average values ± sd.

All samples were recorded in five replicates.

2.7 Statistics

All analyses were performed in triplicates. Color measurements were performed in five replicates. The results are reported as average values ± sd.

3. Results and discussion

3.1 Spectral characterization

a. ^{1}H-NMR data

Most vegetable oils (*e.g*. sunflower, soybean, rapeseed oil) contain predominantly saturated (C16:0, C18:0) and un-saturated (C18:1, C18:2, C18:3) long-chain fatty acids, while milk fat differs substantially in its fatty acid profile, containing significant amounts of short-chain fatty acids (C4:0-C6:0) and medium-chain fatty acids (C8:0-C12:0) [13].

Fig. 2 shows the comparative ^{1}H-NMR spectra of Control, CA-25%-SF and CA-50%-SF samples. As expected, the spectra of the Control sample (containing exclusively milk fat) and those of the cheese analogues are similar in terms of signals. The typical ten signals characteristic of specific structural groups of the acyl chains and the glycerol backbone were comprehensively described in previous publications [27,28].

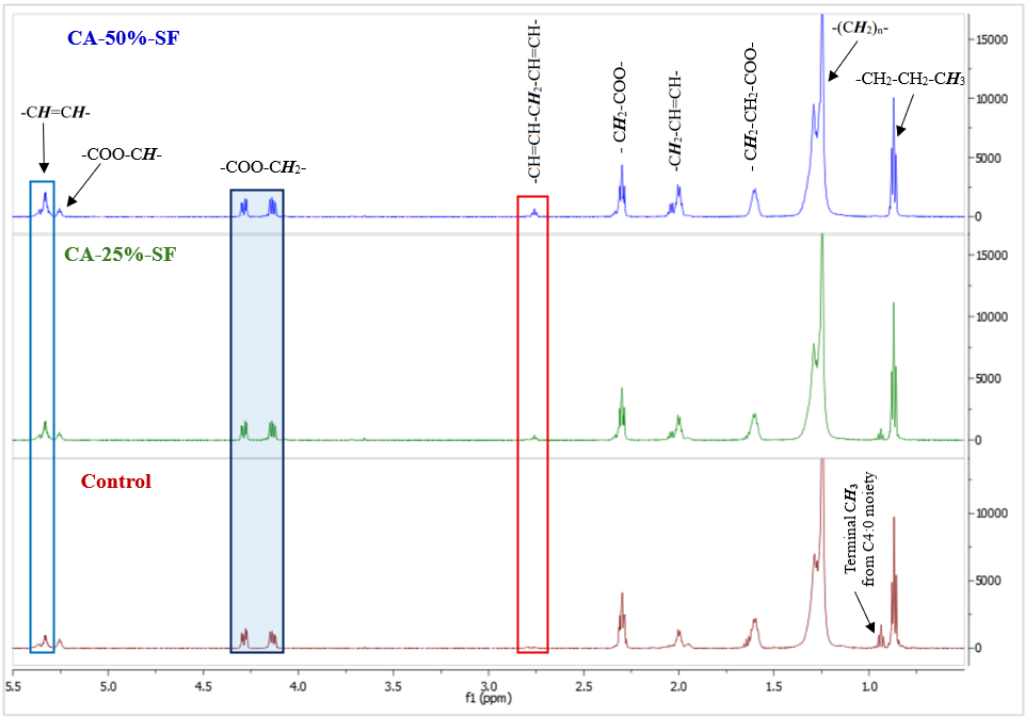


Fig. 2: ^{1}H-NMR spectra of Control, CA-25%-SF and CA-50%-SF samples

As revealed from *Fig. 2*, the addition of fats of non-dairy origin does not necessarily lead to new signals, being mainly reflected in changes of intensity of the existing resonances. Hence, at the same degree of amplification (set on the resonance of the glycerol *sn*-1,3 protons at 4.20 ppm), the intensity of the vinylic protons increases in cheese analogues with the addition of vegetable oils compared to the Control, because of the increased content of (poly)unsaturated fatty acids. In addition, the presence of linoleic acid (C18:2) as the main fatty acid in sunflower oil [29], results in increasing intensities of the multiplet resonance at 2.75 ppm in cheese analogues, since it corresponds to the *bis*-allylic protons. It is noteworthy that *bis*-allylic protons are absent in the Control sample, as milk fat only contains very small amounts (generally less than 3%) poly-unsaturated fatty acids.

On the other hand, the triplet at 0.95 ppm, characteristic of the terminal CH_3 group of the butyric acyl chains, is gradually decreasing, since vegetable oils do not contain short chain fatty acids. As pointed out in a previous paper [13], the 0.95 triplet may be mistaken for linolenic (C18:3) acid in vegetable oil-milk fat mixtures. However, in the case of the CA-25%-SF and CA-50%-SF cheese analogues, the triplet is attributed only to the butyric moiety, because sunflower oil does not generally contain linolenic acid [30].

b. ^{13}C-NMR data

The comparative ^{13}C-NMR spectra of Control, CA-25%-SF and CA-50%-SF samples are presented in *Fig. 3*. It is evident that carbon signals group into five main spectral regions: a) carbonyl atoms region (~175 ppm); b) carbon atoms involved in -C=C- double bonds region (~130 ppm); c) carbon atoms of the ester groups from the glycerol backbone region (~60-70 ppm); d) methylene groups region (~20-35 ppm); e) chain ending methyl groups region (~14 ppm) [29].

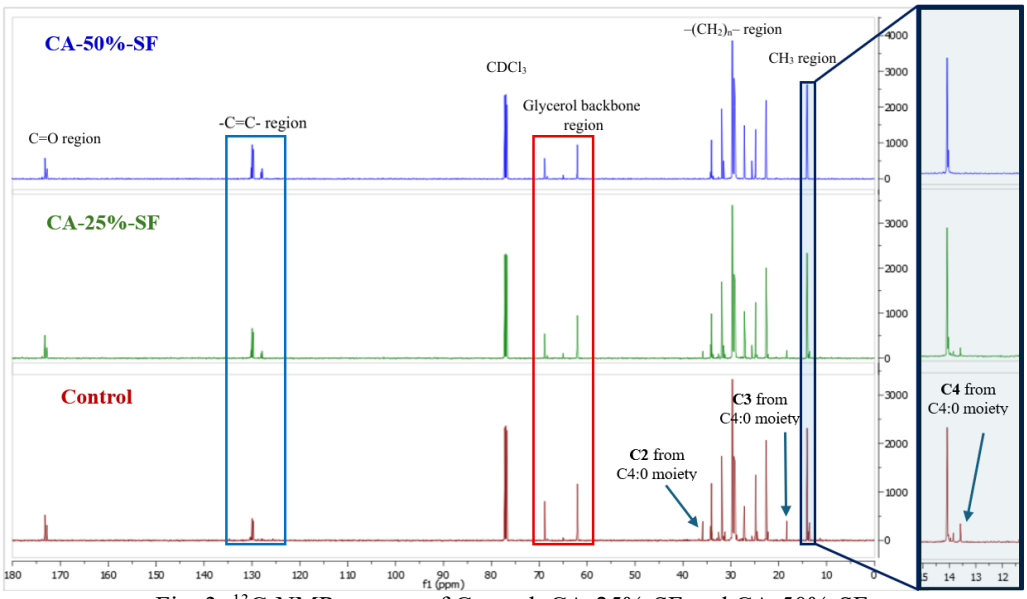


Fig. 3: ^{13}C-NMR spectra of Control, CA-25%-SF and CA-50%-SF samples with a detail on the terminal CH_3 region

In the case of ^{13}C-NMR spectra, it is noteworthy that butyric acid – characteristic of lactic fat – presents distinctive signals, different from the signals of similar C atoms in other fatty acids. Thus C2 (in the vicinity of the ester group) appears at ~35.85 ppm, being more de-shielded compared to similar C atoms in the rest of the acyl moieties; on the other hand, C3 (β position to the ester group) and C4 (terminal methyl group of butyric acid) resonances appear in the spectrum at ~18.25 ppm and ~13.45 ppm, respectively, being shifted up field, compared to the resonances of similar C atoms in other fatty acids. These signals present intensity variations correlated with the addition of vegetable oil, decreasing with increasing oil proportion.

On the other hand, it is important that in the -C=C- region, the intensity of the signals increases as the proportion of sunflower oil increases, due to the increase in the content of (poly)unsaturated fatty acids, especially linoleic acid, which is the main fatty acid in sunflower oil. An interesting aspect can be observed in the region of the C atoms in the glycerol backbone: the intensity of these signals decreases

with the addition of vegetable oils, which is correlated with the decrease of the saponification index and the increase of the fatty acid chain length [31].

3.2 Nutritional characterization

Dairy fat, particularly in the form of butter or high fat content cheese, is rich in saturated fatty acids, which have been traditionally associated with an increased risk of atherosclerosis and cardiovascular disease due to their potential to elevate LDL cholesterol levels. Consequently, it is advisable for adults to limit their intake of dairy fats to mitigate these risks [15]. Several studies have examined the relationship between regular dairy intake – particularly whole milk – and cardiovascular disease (CVD) across diverse populations. Notably, differential associations have been reported among Chinese and British participants, suggesting that the physiological response to dairy consumption may vary according to geographic and ethnic background [32]. In a large population-based cohort from Norwegian counties, where milk consumption is traditionally high, whole milk intake was positively associated with increased risk of CVD and all-cause mortality. In contrast, consumption of low-fat milk appeared to be associated with a reduced risk compared to whole milk [33]. On the other hand, there are also divergent opinions regarding the relationship between dairy fat and the risk of CVD. Thus, a comprehensive study from 2013 analyzed biomarkers of dairy fatty acids and their association with cardiovascular disease risk. The findings indicated that higher intake of dairy fat was associated with a lower risk of cardiovascular disease across diverse populations, including those in Sweden, suggesting that dairy fat intake may not be as detrimental as previously thought [34]. Other authors suggest that, overall, the consumption of milk, yogurt, and cheese—regardless of their fat content—is neutrally associated with the risk of cardiovascular diseases [35]. Therefore, the relationship between whole milk and dairy intake and their potential health risks remains inconclusive and continues to be debated in scientific literature.

However, it is important to note that the overall dietary context matters. Replacing dairy fat with healthier fat sources, such as polyunsaturated fats from plants, has been shown to reduce cardiovascular risk [15]. Therefore, while moderate consumption of dairy fat may not pose significant health risks, it is prudent to limit its intake and focus on a balanced diet rich in unsaturated fats.

The nutritional health indices of cow milk cheese (Control) and its cheese analogues partially substituted with sunflower or rapeseed oils are summarized in *Table 2*. These indices include the atherogenicity index (AI), thrombogenicity index (TI), PUFA/SFA ratio, hypocholesterolemic/hypercholesterolemic ratio (h/H), and the health-promoting index (HPI).

AI estimates the potential of dietary fatty acids to promote the formation of atherosclerotic plaques. Higher values indicate a greater proportion of saturated fatty acids associated with cardiovascular risk [36]. In general, AI values below 1

are considered favorable, while values above 2 indicate higher atherogenic potential [37]. TI reflects the likelihood of clot formation induced by fatty acids. Lower values are more desirable, suggesting a reduced risk of thrombosis, whereas higher values indicate greater thrombogenic potential. Typically, TI values below 1–1.5 are favorable, and values above 3 suggest increased risk [36]. The ratio of polyunsaturated (PUFA) to saturated fatty acids (SFA) indicates the balance between "heart-healthy" and potentially harmful fats. Higher PUFA/SFA ratios are associated with lower cardiovascular risk, with values above 0.4–0.5 generally considered beneficial, and ratios below 0.1 reflecting a less favorable lipid profile [36]. The h/H index represents the balance between fatty acids that lower cholesterol (hypocholesterolemic) and those that raise it (hypercholesterolemic). Higher h/H values indicate a more favorable impact on blood cholesterol. Values above 1 are typically considered beneficial [38]. HPI provides an integrated assessment of the nutritional quality of dietary fats, balancing beneficial and harmful fatty acids. Higher HPI values reflect greater cardiovascular health potential. It has been described as inversely proportional to AI, reinforcing its role as an overall health marker [36].

Table 2

Nutritional health indices

Samples and ingredients	Nutritional health indices*				
	AI	**TI**	**PUFA/SFA**	**h/H**	**HPI**
Sunflower oil	0.08	0.15	5.80	12.42	12.41
Rapeseed oil	0.05	0.07	5.16	18.24	18.51
Control	2.48	4.54	0.05	0.53	0.40
CA-50%-SF	0.73	1.34	0.87	2.13	1.37
CA-25%-SF	1.34	2.47	0.35	1.12	0.74
CA-50%-R	0.67	0.94	0.45	2.36	1.50

* Computed based on the fatty acid profiles reported in literature [29,30,39].
** Non-dimentional, computed from GC data based on the formulas in literature [37,40,41].
*** AI = atherogenicity index; TI = thrombogenicity index; PUFA/SFA = poly-unsaturated fatty acids/ saturated fatty acids; h/H – hypocholesterolemic/ hypercholesterolemic index; HPI = health promoting index.

As reflected in *Table 2*, the Control sample showed the highest atherogenicity (AI = 2.48) and thrombogenicity (TI = 4.54) indices, along with a very low PUFA/SFA ratio (0.05), indicating a lipid profile dominated by saturated fatty acids. Consequently, the h/H (0.53) and HPI (0.40) indices suggest a relatively low cardiovascular health potential. These results are in agreement with similar data reported in the literature on cheese lipids characterization [41,42].

Substitution of milk fat with sunflower oil markedly improved the lipid profile. For example, AI and TI decreased to 0.73 and 1.34 in CA-50%-SF, while CA-25%-SF showed intermediate values (AI = 1.34, TI = 2.47). The PUFA/SFA ratio increased with the degree of substitution, reaching 0.87 in CA-50%-SF, reflecting higher polyunsaturated fatty acid content. Similarly, h/H and HPI indices improved proportionally, indicating enhanced health-promoting potential.

The rapeseed oil-based analogue (CA-50%-R) exhibited the most favorable profile, with AI = 0.67, TI = 0.94, PUFA/SFA = 0.45, h/H = 2.36, and HPI = 1.50. This suggests that rapeseed oil contributes to a more balanced fatty acid composition, likely due to a higher proportion of mono- and polyunsaturated fatty acids compared to sunflower oil.

Overall, partial replacement of milk fat with vegetable oils effectively reduces atherogenic and thrombogenic risks and enhances lipid-related health indices. The type of oil and the level of substitution are key factors, with rapeseed oil showing superior benefits. These findings highlight the potential of vegetable oil-enriched cheese analogues as healthier alternatives to traditional cow milk cheese. The improvement in lipid-related nutritional indices observed in the present study is consistent with previous research demonstrating the beneficial effects of substituting dairy fat with vegetable oils in cheese matrices. For instance, replacement of 50–100% of milk fat with canola or olive oils in Iranian white brined cheese led to a decrease in saturated fatty acids and a concomitant increase in unsaturated fatty acids, thereby improving the nutritional quality of the lipid fraction and suggesting lower atherogenicity and thrombogenicity potential [43]. Similarly, in an experimental study on rats, partial substitution of dairy fat in soft-ripened cheese with a blend of sunflower and soybean oils resulted in a significant reduction in LDL cholesterol (–31%) and an increase in HDL cholesterol (+11%), leading to an improved LDL/HDL ratio (–39%) [44]. These findings confirm that the incorporation of vegetable oils into cheese formulations contributes to a more favorable lipid profile, which aligns with the reductions in AI and TI and the improvements in h/H and HPI observed in our study.

3.3 Rheological evaluation

To assess how the addition of vegetable oils has influenced the mechanical properties of cheese analogues, their rheological analysis was necessary.

Fig. 4 shows the variation of rheological parameters G' and G" as a function of frequency across the samples investigated. As reflected, all four samples have similar rheological behavior. According to the chart, the cheese analogue enriched with rapeseed oil (CA-50%-R) has the highest G' and G" when compared with the conventional cheese (Control, 100% cow milk). It also appears that this analogue has the most pronounced solid behavior of the three.

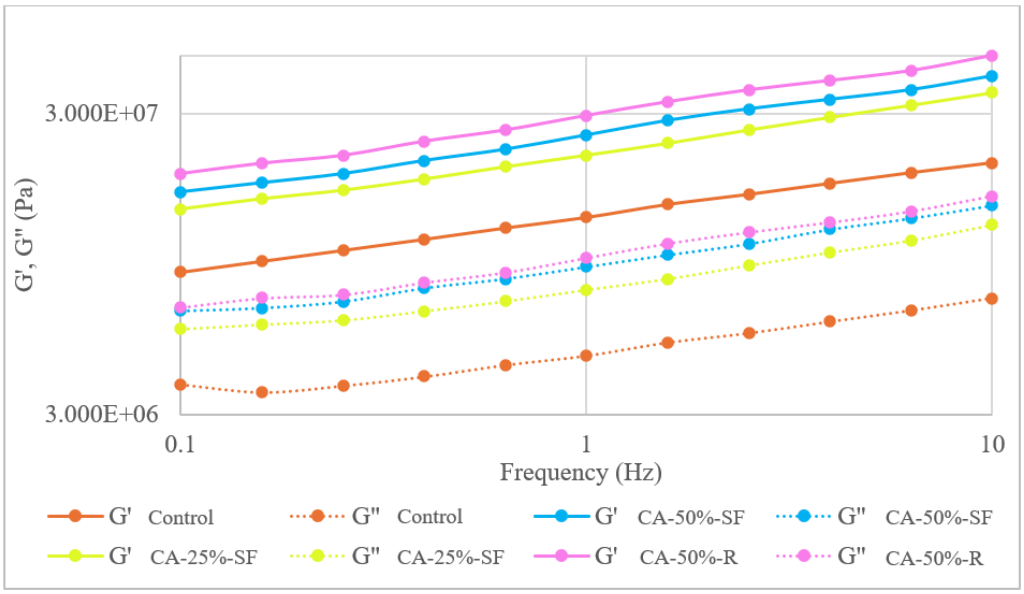


Fig. 4: Variation of rheological parameters G' and G" as a function of frequency for the investigated samples (Control, CA-25%-SF, CA-50%-SF and CA-50%-R)

The elastic modulus G' is higher than the viscous modulus G" across all samples, which means that both the conventional cheese and the analogues enriched with vegetable oils behave like solid gels. The increase of G' and G" with frequency shows that the internal structure of all samples remains solid at rapid deformations such as chewing.

The Control sample has the lowest elastic modulus (G'), which increases with the increase in the amount of oil added (G' Control < G' CA-25%-SF < G' CA-50%-SF < G' CA-50%-R). It can also be observed that the sample with 50% rapeseed oil has the highest elastic modulus. This is due to the different chemical composition of the two oils, rapeseed oil having a higher content of unsaturated fatty acids – 92.70 g/100g compared to sunflower oil – 86.72 g/100g [29].

Although from the visual and tactile viewpoints, the obtained cheese analogues present a softer, creamier texture compared to the conventional cheese sample, this does not automatically imply fluid behavior from a rheological perspective. The results suggest that the oil acts as an active filler, being incorporated into the protein matrix as protein-stabilized droplets. This incorporation leads to an increase in the elastic modulus (G') and a decrease in the viscous modulus (G"), indicating a more rigid and less deformable structure. Strong interactions between the oil and the protein matrix contribute to gel cohesion and stability, with the oil behaving as an integrated component within the gel network. These findings indicate that the addition of vegetable oils to cheese analogues can significantly influence the viscoelastic properties of the product, functioning as an active filler within the protein network. The results obtained are consistent with data

on cheese analogues based on legume proteins and sunflower oil [45] and from other vegetable oils [23].

3.4 Color characterization

The color analysis was performed for each sample using the CIE L*a*b* color space as a set of chromatic characteristics. This model, developed by the International Commission on Illumination (CIE), is considered one of the most comprehensive color representation systems, due to its high correlation with human visual perception. The CIE L*a*b* system separates the chromatic components into a luminance axis (L*), where the value 0 corresponds to black and 100 to white, and two color axes: a* (green-red) and b* (blue-yellow), thus providing an objective and accurate color analysis in digital images [46].

Table 3

Color characterization of the investigated samples

Color parameter	Control	CA-50%-SF	CA-25%-SF	CA-50%-R
L*	92.54±3.25	90.79±4.13	91.28±2.48	91.25±3.62
a*	-2.76±0.21	-2.98±0.14	-1.93±0.34	-1.83±0.18
b*	6.45±0.36	9.14±0.54	7.63±0.48	6.03±0.42
C*	7.02±0.62	9.62±0.60	7.88±0.41	6.37±0.48
H^0	180.99±3.60	170.65±4.16	177.57±4.26	179.05±4.47
ΔE	-	6.46±3.79	5.10±0.31	5.82±0.45

Values represent the average ± sd across 5 replicates

Color parameters (L*, a*, b*) of the Control cheese and the analogues enriched with sunflower or rapeseed oils are presented in *Table 3*. Lightness values (L*) ranged from 90.79 to 92.54 across all samples, indicating a very bright appearance, typical of fresh cheeses with high moisture content [47]. The slight decrease in L* for sunflower- and rapeseed-enriched samples compared to the Control suggests a minor reduction in brightness, although differences were not visually significant.

The a* coordinate, representing the green–red axis, showed negative values in all cases (−2.98 to −1.83), consistent with the slightly greenish hue usually associated with fresh dairy products. The more negative a* values for sunflower oil samples (−2.98 and −1.93) may be linked to chlorophyll-related pigments naturally present in the oil.

The b* coordinate (yellow–blue axis) revealed the most pronounced variations. Sunflower oil analogues displayed higher b* values (9.14 and 7.63) compared to the Control (6.45), indicating an intensified yellow tint. This can be attributed to carotenoid pigments in sunflower oil. Conversely, the rapeseed oil

analogue (6.03) showed a lower b* value, closer to the Control, suggesting a less marked yellowish hue.

Overall, the incorporation of vegetable oils influenced chromatic attributes, particularly in the yellow–blue axis, without compromising the visual characteristics of the cheeses. Sunflower oil imparted a stronger yellowish tone, while rapeseed oil maintained a more neutral color profile. These findings align with the compositional differences in pigments between the two oils and confirm that vegetable oil substitution has measurable but acceptable effects on cheese color.

Comparable findings have been reported in the literature: sunflower oil–enriched cheese analogues show increased yellowness due to intrinsic carotenoids (e.g., in spreadable cheese systems) [47]. Similarly, partial substitution of milk fat with canola (rapeseed) oil in fresh soft cheese analogues resulted in minimal impact on color parameters while improving nutritional quality [48]. These observations align well with our data and support the predictable, compositional basis of chromatic shifts.

4. Conclusions

The present study demonstrated that partial substitution of milk fat with sunflower and rapeseed oils leads to cheese analogues with improved nutritional and physicochemical characteristics. NMR spectroscopy confirmed the expected changes in fatty acid composition, with increased signals related to polyunsaturated fatty acids in vegetable oil-enriched samples. Nutritional health indices (AI, TI, PUFA/SFA, h/H, HPI) improved markedly, particularly in the rapeseed oil analogue (CA-50%-R), which exhibited the most balanced lipid profile. Rheological analysis indicated that the addition of vegetable oils enhanced the elastic modulus, suggesting the oils were successfully incorporated into the protein matrix as active fillers, reinforcing gel structure. Colorimetric evaluation highlighted slight but acceptable changes in brightness and chromatic parameters, with sunflower oil imparting a more yellowish tint. Overall, the incorporation of vegetable oils into cheese formulations represents a viable strategy for producing healthier dairy alternatives with preserved technological and sensory functionality, offering promising applications in the functional foods sector.

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