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RESEARCH ARTICLE

Impact of phytosterols addition on physico-chemical, sensory, fatty acid profile and microstructural properties of processed cheese spread

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Abstract: A functional processed cheese spread (PCS) was developed by incorporating three levels of phytosterols—2%, 3%, and 4%—separately during its manufacture. As the level of phytosterols addition increased, the water activity (a_w) at 3% and 4% levels gradually increased, while titratable acidity significantly decreased (p< 0.05) compared to the control (CPCS). At 4% phytosterols addition, a powdery flavor, lack of cheese flavor, and reduced spreadability were observed. At higher levels of phytosterols addition (3% and 4%), panelists detected a slightly coarse texture and sandiness on the tongue. A significant (p<0.05) decrease in the total sensory score was noted at phytosterols levels of 3% and above. Based on these findings, 2% phytosterols addition was optimized for PCS. In phytosterol-incorporated processed cheese spread (PPCS), total saturated fatty acid content increased, while total unsaturated fatty acid content decreased compared to CPCS. The scanning electron micrograph of PPCS revealed that insoluble phytosterols formed a coarse network, significantly disrupting the cheese matrix.

Keywords: Processed cheese spread; Phytosterols; Sensory; Microstructure; Fatty acid profile

Introduction

In recent years, consumers have begun to view food not only as a source of energy but also as a means to achieve health benefits. It is estimated that up to 80% of coronary heart disease, 90% of type 2 diabetes, and one-third of cancers can be prevented by adopting a healthier lifestyle, including dietary modifications (Govori et al. 2024). However, dietary habits are notoriously difficult to change and even more challenging to sustain, even when their positive health effects are well known. In this context, functional foods play a crucial role. Consequently, the focus of research among nutritionists and food companies has shifted toward the enrichment of conventional foods with functional ingredients (Giriand Rao2025)

Among various commercially available functional ingredients, phytosterols have been widely incorporated into different food products. Plant-derived phytosterols, due to their structural similarity to cholesterol, reduce intestinal absorption of both dietary and endogenously produced cholesterol without lowering HDL cholesterol levels. It is recommended that 2 g of plant sterols per day be consumed to achieve cholesterol-lowering effects (Zio et al. 2024), equivalent reductions may also be possible with smaller doses.

Among dairy products, cheese has been a part of human diet since ancient times. Cheese is valued for its high nutritional content, portability, long shelf life, and rich in fats, proteins, and minerals. Cheese has a high casein content, which serves as a reservoir for several bioactive peptides. These peptides exhibit various biological activities, including anti-cariogenic, anti-hypertensive, anti-oxidative, and opioid-like effects (Nourmohammadi and Mahoonak, 2019). In addition to bioactive peptides, cheese is an excellent source of highly bioavailable calcium(Sohail et al. 2023). Cheese contains significant amounts of conjugated linoleic acid (CLA), which has been associated with body fat reduction (Giri et al. 2015), cancer inhibition, and immune modulation. The CLA content further increases during the manufacture of processed cheese from natural cheese (Parodi, 2020). This increase mainly occurs during processing, where heating and anaerobic conditions convert linoleic acid into CLA isomers. CLA is generally expressed on a fat basis (mg/g fat), with cis-9, trans-11 CLA being the predominant type. Moreover, contrary to common misconceptions, milk fat in cheese has been reported to have no adverse effects on serum cholesterol levels. A study revealed that cheese consumption was associated with lower serum cholesterol levels compared to butter consumption, despite both containing identical amounts of fat (Timon et al. 2020).

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India is not traditionally a cheese-consuming nation, with paneer being the preferred fresh cheese. However, cheese consumption has been rising in recent years, particularly in the industrial and retail sectors. The Indian cheese market is largely dominated by processed cheese, which accounts for about 60% of the total share due to its affordability, consistency, and mild flavor suited to local preferences. Cheese spreads make up nearly 30% of the processed cheese segment, while mozzarella and flavored cheeses are also gaining popularity. This trend highlights the rapid growth of processed cheese as the major driver of India's expanding cheese market.

Thus, the objective of the present study was to enhance the functionality of PCS by incorporating phytosterols. To determine the optimum level of phytosterols addition, three levels—2%, 3%, and 4%—were incorporated during the manufacture of PCS. The PCS samples were evaluated for various physicochemical and sensory characteristics and compared with a control processed cheese spread (CPCS) sample that did not contain phytosterols.

Materials and Methods

Raw Materials and Ingredients

Cheddar Cheese

For the manufacture of PCS, both young (~1½-month ripened) and old (~6-month ripened) varieties of Cheddar cheese were used. Cheddar cheeses of both ages was obtained from the Experimental Dairy of the National Dairy Research Institute, Karnal, Haryana, India. The quality of the Cheddar cheese used for the preparation of PCS is mentioned in Table 1(Giri et al. 2017).

Phytosterols

Phytosterols (extracted from soybean; Phytosterols 95%, Bio-gen Extracts Pvt. Ltd., Bangalore) were used to prepare PPCS. The phytosterols extract contained total phytosterols (95.1%), including β-sitosterol (41.0%), stigmasterol (28.8%), campesterol (25.0%), and brassicosterol (0.30%). A mixture of these soybean-derived phytosterols, as supplied by the manufacturer, was utilized in the study.

Other Ingredients

Iodized common salt (TATA Salt, Tata Chemicals Ltd., Mumbai) was procured from the local market for the preparation of PCS. Tri-sodium citrate (Posy PharmachemPvt. Ltd., Ahmedabad) was used as an emulsifying salt in PCS manufacturing.

Manufacture of Phytosterol-Incorporated Processed Cheese Spread

A blend of Cheddar cheese (25% old Cheddar cheese and 75% young Cheddar cheese) was used for the preparation of PCS. The amounts of different ingredients, such as water, salt, and trisodium citrate (as an emulsifier), were calculated based on the final product composition. In this investigation, each batch of PCS weighed 1 kg.

The young and old Cheddar cheeses were cleaned, quartered, and grated. A cheese processing vessel (Stephy®, Stephy Industries, New Delhi) was used for the manufacture of PCS. Initially, a portion of the grated cheese was placed into the cheese processing cooker, and the calculated amounts of salt and emulsifier were mixed into the required amount of water before being added to the cooker. The remaining grated cheese was then added, and the contents in the vessel were heated while being continuously stirred and scraped with a wooden ladle.

When the cheese mixture became semi-solid and homogeneous, phytosterols powder was incorporated. To ensure even dispersion, the phytosterols powder was first mixed with warm water (50°C) using a running blender (Mincer Blade, Type-H-100, RPM-12000, Lee Handy™; Lumix Appliances, Ambala City) for 3 minutes. After straining, the phytosterols mixture was added

Table 1: Quality of Cheddar cheese used for preparation of processed cheese spread

Parameters	Young Cheddar cheese (~1½ month)	Old Cheddar cheese (~6 months)
Moisture (%)	39.5	37.0
Total fat (%)	29.0	30.0
Free fatty acid (μg/g)	17.8	20.9
Total protein (%)	24.8	25.9
Soluble protein (%)	6.3	11.1
Ripening index	25.4	42.9
Salt (%)	3.0	3.1
Ash (%)	3.5	3.7
Acidity (% lactic acid)	1.03	0.82
pH	5.3	5.8

Cheese blend (Young cheese:Old cheese::3:1) Ripening index = 29.8

to the cheese spread and thoroughly mixed. The entire mixture was heated to 85°C for 3–5 minutes with continuous agitation.

After heating, the processed cheese spread was immediately transferred into sterile sample containers (50 mL capacity; made of high-density polyethylene with a polypropylene lid; Tarsons Products Pvt. Ltd., Kolkata). The PCS was allowed to cool to room temperature and then stored under refrigeration (4 ± 1°C) for further analysis (Giri and Kanawjia 2013a, b; Giri et al. 2014a, b).

Analytical Methods

Physico-chemical Attributes

The moisture content of Cheddar cheese and PCS was determined using the standard gravimetric method, as described in IS: SP: 18 (Part XI) 1981. The fat content in both Cheddar cheese and PCS was determined by the Gerber method, as described in AOAC (1990). The total protein content was estimated using the Micro-Kjeldahl method (AOAC, 1992).

The salt and total ash contents in Cheddar cheese and PCS samples were estimated according to the procedure outlined in IS: SP: 18 (Part XI)1981. The titratable acidity of PCS was determined following the AOAC (1995) method.

For pH determination, 10 g of Cheddar cheese or PCS was mixed with 10 mL of deionized water to prepare a slurry (Møller et al. 2013). The pH of the slurry was measured using a microprocessor-controlled pH analyzer (Version I, Labindia, New Delhi) with a combined glass electrode. The pH meter was calibrated using buffer solutions at pH 9.2 and 4.0 and standardized at pH 7.0 before measurement.

The water activity of PCS was measured using a water activity meter (Aqua Lab, Model Series 3TE, Decagon Devices, WA, USA). Before measurement, the samples were tempered at 25°C (Giri et al. 2017; Badola et al. 2018).

Fat from cheese samples was extracted following the AOAC (2011) method and converted to fatty acid methyl esters (FAMEs) by trans-esterification using methanolic KOH. The FAMEs were analyzed using Gas-Liquid Chromatography (GC Model 5765, NUCON, New Delhi) equipped with a flame ionization detector (FID) and a capillary column (30 m × 0.25 mm × 0.25 µm, DB-23, Agilent Technologies). The injector and detector temperatures were maintained at 250°C, while the oven was programmed from 140°C (held 5 min) to 230°C at 4°C/min. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. Peaks were identified by comparison with a mixture of standard fatty acids (Sigma-Aldrich, USA).

Phytosterols in PPCS were extracted by saponification with ethanolic KOH, followed by extraction with hexane. The unsaponifiable matter was derivatized to trimethylsilyl ethers and quantified using GC-FID under the same operating conditions as above, according to the methods of Sabir et al. (2003) and Attarde et al. (2010).

Sensory Analysis

Sensory evaluation of processed cheese spreads was carried out using a descriptive sensory scoring method performed by 10 trained panellists from the institution. The evaluated attributes included flavour, body and texture, colour and appearance, and spreadability. The maximum scores for each attribute were as follows: Flavour – 45, Body and Texture – 30, Colour and Appearance – 10, and Spreadability – 15, with a total sensory score of 100. The analysis was conducted in a sensory laboratory designed according to ISO standards (ISO 8589:1985).

Panellists were provided with water and bland crackers for palate cleansing between samples. All samples (~50 g) were randomly coded using three-digit numbers. Each panellist received the samples at 20°C in white 50 mL HDPE sample containers, always 2 h before or after meals.

Microstructure

Scanning electron microscopy (SEM) was used to determine the microstructure of cheese spread samples. Samples were prepared according to the method described by Giri et al. (2017) and Karami et al. (2009). For this, cheese spreads were spread on a flat and smooth surface and, using a shaving blade, cut into approximately 5–6 mm^3 blocks. These blocks were immersed in a fixative solution (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M sodium phosphate buffer, pH 7.2) for 3 h and then freeze-dried.

To prevent damage to fat globules, cheese spread samples were not washed using an ethanol/water combination or acetone. The samples were then cut into approximately 1 mm thick sections, mounted on aluminum stubs using double-sided sticky carbon-based tape, and coated with gold for 200 seconds using a sputter coater (Polaron SC7640, East Sussex, UK). The prepared samples were examined under an SEM (Carl Zeiss EVO 40, Oberkochen, Germany) at 20 kV.

Statistical Analysis

The levels of inulin were analyzed using one-way ANOVA with the IBM SPSS Statistics 20 software package. Data are presented as means ± standard error (SE). When significant differences ($p < 0.05$) were observed, individual means were compared using Tukey's post hoc multiple comparison test (Giri et al. 2014).

Results and Discussion

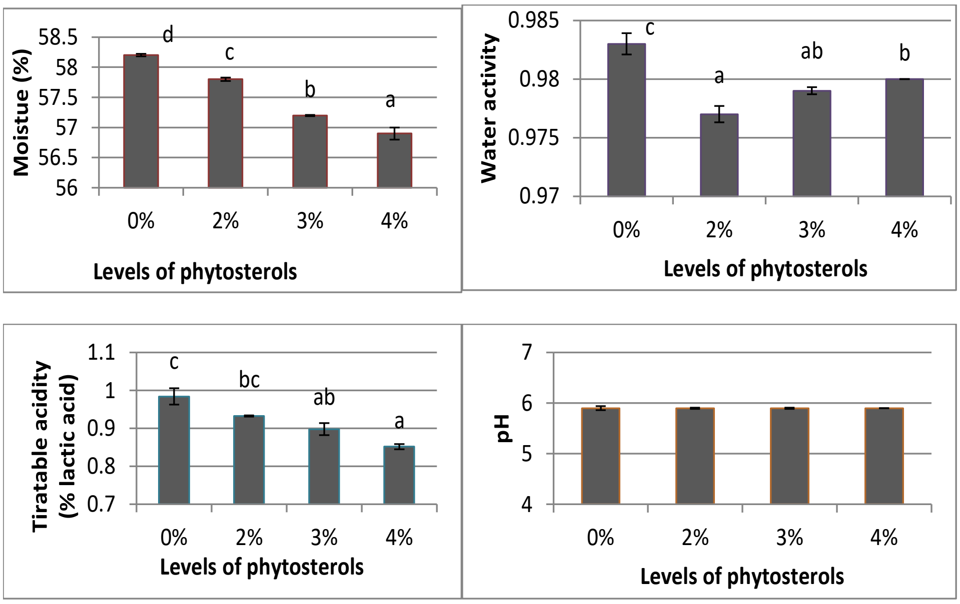
Physico-Chemical Characteristics

Fig. 1 reveals that phytosterols addition followed an inverse relationship with the moisture content in the product. As the level of phytosterols addition increased from 0% to 4% in the cheese spread, the moisture percentage significantly decreased ($p < 0.05$) from 58.2% to 56.9%. This reduction in moisture content was due to the proportionate increase in total solids in the cheese spread.

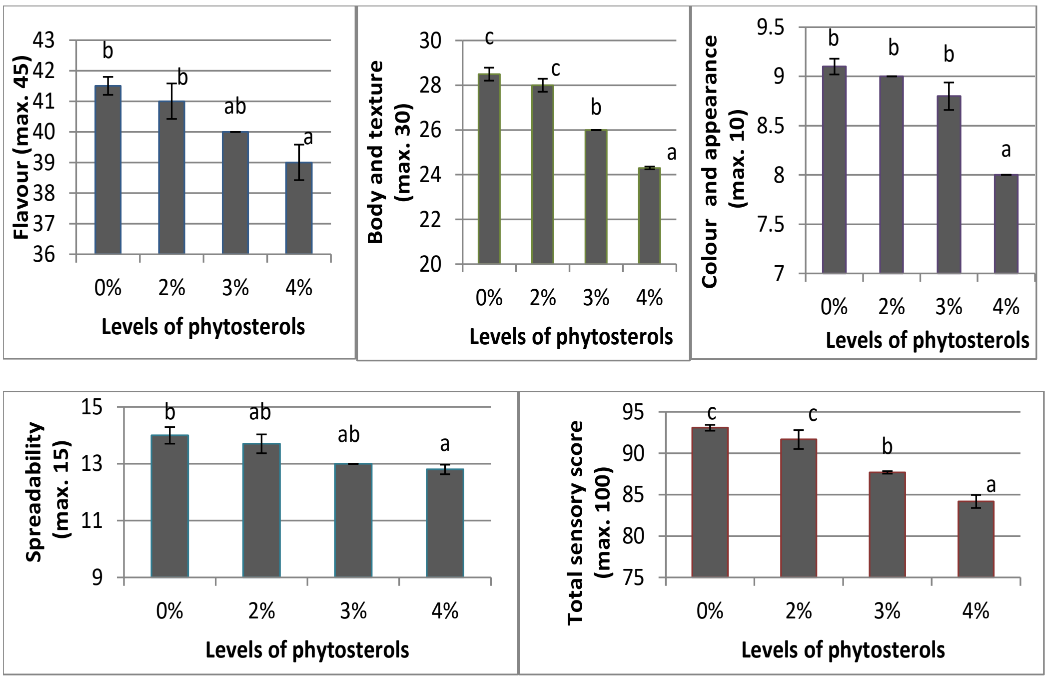
Due to the decrease in moisture content, the a_w value significantly decreased ($p < 0.05$) at 2% phytosterols addition from 0.983 to 0.977 compared to the control. However, at higher levels (3% and 4%) of phytosterols addition, a_w gradually increased, reaching 0.980 at 4% phytosterols addition. This could be attributed to increased water expulsion from the cheese matrix due to the interaction of phytosterols with the cheese structure.

The initial titratable acidity of the control PCS was found to be 0.984. Phytosterols addition up to 2% did not cause a significant change in the titratable acidity of the cheese spread. However, at 3% and beyond, it decreased significantly ($p < 0.05$) compared to the control, reaching 0.852 at 4% phytosterols addition. Despite this decrease in titratable acidity, the pH of the cheese spread

Fig. 1 Effect of different levels of phytosterols on different physico-chemical (1A)and sensory (1B) characteristics of processed cheese spread; n=5;Different small alphabets indicate significantly different (p<0.05); Vertical bars indicate standard errors



1A



1B

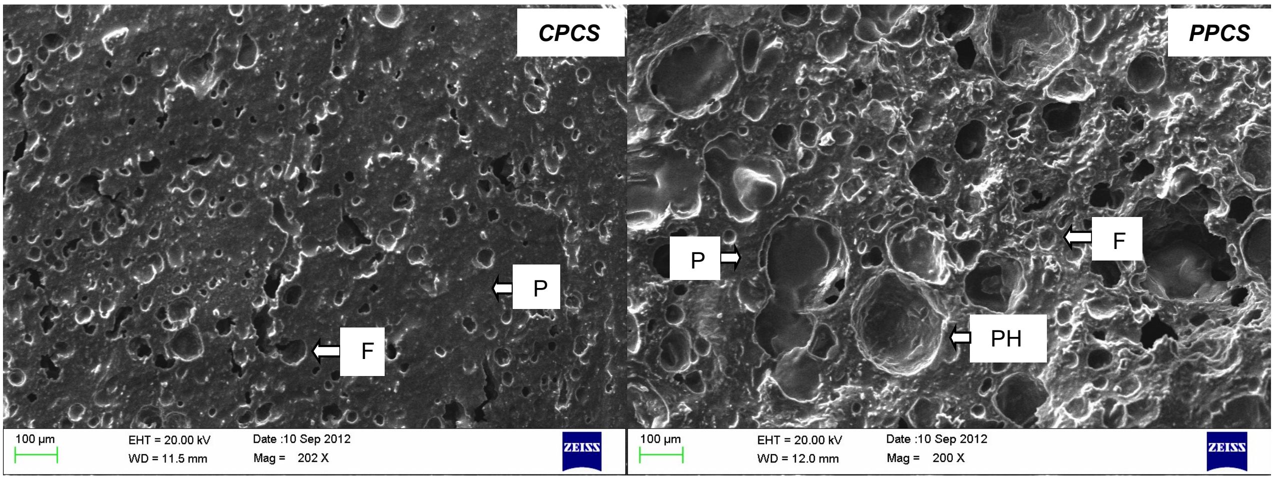


Fig. 2 Scanning Electron Micrograph of control processed cheese spread (CPCS) (Giri et al. 2017) and phytosterols incorporated processed cheese spread (PPCS); P: Protein matrix; F: Fat globule; PH: Phytosterols

remained unchanged at 5.9 due to the high buffering capacity of the cheese spread.

The initial titratable acidity of the control PCS was 0.984. Phytosterol addition up to 2% did not significantly affect the titratable acidity of the cheese spread. However, at 3% and beyond, the acidity decreased significantly ($p < 0.05$) compared to the control, reaching 0.852 at 4% phytosterol addition. This reduction may be attributed to hydrophobic and matrix-binding interactions of phytosterols with organic acids, which lower the availability of dissociable acid groups for titration. Despite this decrease in titratable acidity, the pH of the cheese spread remained unchanged at 5.9 due to the buffering action of casein–phosphate–citrate salts system in the processed cheese matrix.

Sensory Characteristics

Flavour

The effect of different levels of phytosterols on the sensory characteristics of PCS is presented in Fig. 1. The flavour score decreased from 41.5 to 39.0 as phytosterols addition increased from 0% to 4%. Although up to 3% phytosterols addition, the flavour score did not change significantly ($p> 0.05$), a significant ($p< 0.05$) reduction was observed beyond this level. Sensory panellists noted a typical powdery flavour in cheese spread with 4% phytosterols and reported a lack of characteristic cheese flavour at higher phytosterols levels.

Body and Texture

As the level of phytosterols increased from 0% to 4% in the cheese spread, the body and texture score decreased from 28.5 to 24.3. At 2% phytosterols addition, there was no significant ($p> 0.05$) difference in the body and texture score compared to the control. However, beyond this level, the score significantly decreased ($p< 0.05$). Phytosterols are insoluble in water and only partially soluble in some non-polar solvents. At higher concentrations, they remained insoluble in the cheese matrix, leading to a coarse and sandy mouthfeel. This defect was not observed at lower levels (2%). Additionally, higher levels of phytosterols addition significantly ($p< 0.05$) affected textural attributes, increasing firmness and work of shear while decreasing work of adhesion compared to the control, which contributed to the lower body and texture score.

Colour and Appearance

The colour and appearance score decreased from 9.1 to 8.0 as the level of phytosterols addition increased from 0% to 4%. However, up to 3% phytosterols addition, there was no significant ($p> 0.05$) difference compared to the control. At 4%, a significant ($p< 0.05$) decrease was observed. The reduced solubility of phytosterols in the cheese matrix led to a lack of the characteristic yellowish colour and smooth appearance.

Spreadability

The spreadability score decreased from 14.0 to 12.8 as the phytosterols level increased from 0% to 4%. Up to 3% phytosterols addition, there was no significant ($p> 0.05$) difference

Table 2 Proximate composition of phytosterols incorporated (PPCS) along with control (CPCS) processed cheese spread

Constituents (%)	CPCS	PPCS
Moisture	58.2	57.8
Fat	19.0	19.0
Protein	17.4	16.9
Salt	2.0	2.0
Ash	3.4	3.3
Phytosterols	-	1.84

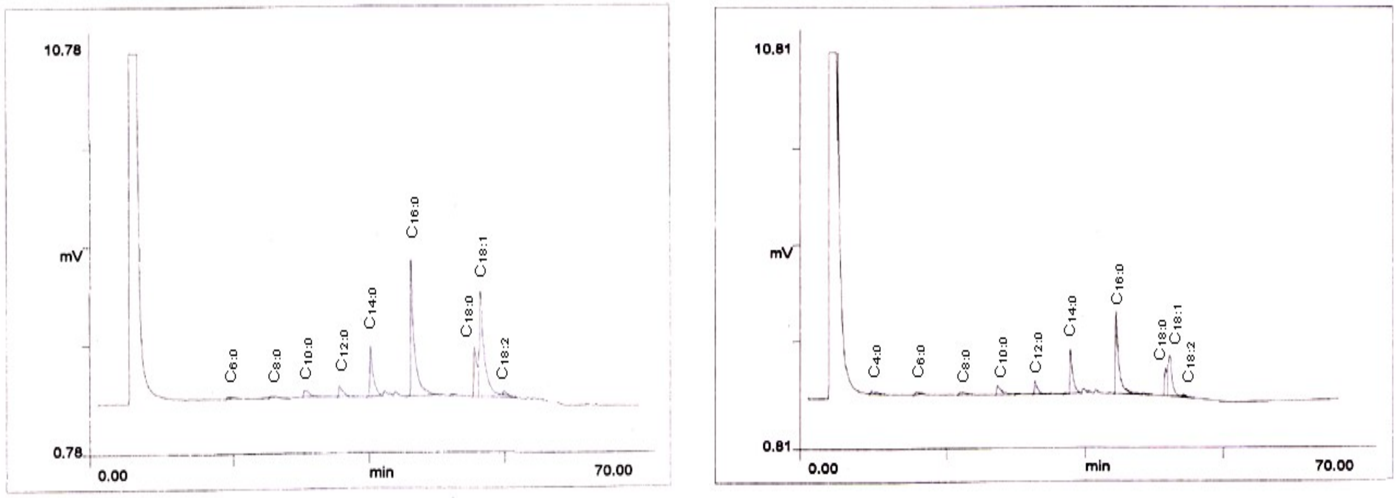


Fig. 3 Gas chromatogram of fatty acids present in control (A) and processed cheese spread added with phytosterols (B)

compared to the control. However, at 4%, the spreadability score significantly decreased ($p < 0.05$), primarily due to reduced softness caused by decreased moisture content and increased graininess from the higher concentration of insoluble phytosterols.

Total Sensory Score

As the level of phytosterols addition increased from 0% to 4%, a gradual decrease in total sensory score from 93.1 to 84.2 was observed. At 2% phytosterols addition, sensory panellists did not report any significant ($p > 0.05$) change in total sensory score compared to the control. However, at 3% and beyond, a significant ($p < 0.05$) reduction was noted. The primary reasons for the decreased sensory score at higher phytosterols levels were perceivable powdery flavour, lack of cheese flavour, increased hardness, coarseness, and poor spreadability. Panellists reported that the cheese spread with 2% phytosterols addition was comparable to the control, making it the optimized formulation in this study.

Analysis of Optimized Product

Proximate Composition

The proximate composition of the developed phytosterol-incorporated cheese spread is presented in Table 2. Compared to the control, phytosterols addition led to a decrease in moisture, protein, and ash percentages, while fat and salt percentages remained unchanged. The final product (PPCS) contained 1.84% phytosterols. The unaltered fat content, despite phytosterols being fatty and waxy, could be attributed to their poor solubility in the lipid phase and tendency to remain dispersed in the protein–carbohydrate matrix, which prevents their detection as milk lipids in standard fat analysis.

Table 3 Fatty acid composition of phytosterols incorporated (PPCS) along with control (CPCS) processed cheese spread

Fatty acids	% Fatty acid present in product	
	CPCS	PPCS
Butyric ($C_{4:0}$)	ND	0.36
Caproic ($C_{6:0}$)	0.07	0.18
Caprylic ($C_{8:0}$)	0.13	0.39
Capric ($C_{10:0}$)	0.50	0.95
Lauric ($C_{12:0}$)	0.58	0.94
Myristic ($C_{14:0}$)	2.09	2.98
Palmitic ($C_{16:0}$)	6.31	6.65
Stearic ($C_{18:0}$)	1.98	1.43
Oleic ($C_{18:1}$)	7.04	4.51
Linoleic ($C_{18:2}$)	0.17	0.13

Fatty Acid Profile Analysis

The addition of phytosterols altered the fatty acid composition, as shown in the gas chromatograms (Fig. 3) and Table 3. CPCS contained a notable amount of linoleic acid (8.95 mg/g fat or 0.17% of the product), which may be attributed to the use of milk fat fractions richer in C18:2, along with anaerobic heat treatment that preserves it as a substrate for conjugated linoleic acid (CLA) formation. Similar studies have reported an increase in CLA during processed cheese production (Govari and Vareltzis 2025), supporting the hypothesis that under anaerobic conditions, CLA isomers can be produced via auto-oxidation of linoleic acid during heating (Giri et al. 2017; Gong et al. 2019).

Phytosterols addition slightly reduced linoleic acid content to 0.13%. Furthermore, the percentages of major fatty acids such as butyric, caproic, caprylic, capric, lauric, myristic, and palmitic acids increased, while stearic, oleic, and linoleic acid percentages decreased. The observed shift in fatty acid profile on phytosterol addition can be attributed to matrix–lipid interactions and differential stability of fatty acids during processing. Phytosterols are insoluble and tend to disrupt the cheese fat–protein matrix, which favors the retention of short- and medium-chain saturated fatty acids (C4:0–C16:0) while reducing the relative proportion of long-chain unsaturated fatty acids. Moreover, unsaturated fatty acids such as oleic and linoleic acid are more prone to oxidative and thermal degradation under heating, especially in the semi-anaerobic conditions of processed cheese manufacture, leading to their relative decline. Consequently, the decrease in unsaturated fatty acids manifested as an apparent increase in total saturated fatty acid content in the phytosterol-incorporated cheese spread (Li et al. 2024). Overall, phytosterols addition increased total saturated fatty acid (SFA) content (from 11.66% to 13.88%) and decreased total unsaturated fatty acid content (from 7.21% to 4.64%). Among unsaturated fatty acids, monounsaturated fatty acids decreased from 7.04% to 4.51%, while polyunsaturated fatty acids decreased from 0.17% to 0.13%. The increase in total SFA after phytosterol addition can be explained by both compositional and analytical factors. Phytosterols are insoluble, hydrophobic compounds that interact differently with the lipid phase of the cheese matrix. Their incorporation likely altered fat partitioning and solubility, favoring the retention and detection of short- and medium-chain saturated fatty acids (e.g., butyric, caproic, caprylic, capric, lauric, myristic, and palmitic acids), which all showed increases in PPCS. In contrast, unsaturated fatty acids such as oleic and linoleic acid decreased, possibly due to reduced stability and higher susceptibility to oxidative/thermal degradation during heating under semi-anaerobic conditions. This selective reduction in unsaturated fatty acids resulted in a relative increase in total SFA proportion in the final product (Zhuang et al. 2022).

Microstructure Analysis

The microstructure of CPCS and PPCS was compared using Scanning Electron Microscopy (SEM) (Fig. 2). In CPCS, fat droplets were uniformly distributed, with sizes ranging from 18–60 µm, which is consistent with the typical microstructure of processed cheese products (Fox et al. 2017; Giri et al. 2017; Hill and Ferrer 2022; Deshwal et al. 2024). Smaller fat globules generally indicate a more effective fat emulsion (Truong et al. 2016; Hussain et al. 2017). However, due to manual stirring, PCS showed some irregularly shaped and larger fat droplets, similar to observations made by Trivedi et al. (2008).

In PPCS, SEM images revealed that insoluble phytosterols appeared as large particles (100–230 µm) within the cheese protein matrix. The microstructure of PPCS exhibited a coarse network, significantly disrupting the cheese matrix. The increased space occupied by phytosterols caused milk fat globules to cluster together. These microstructural differences correlated with the observed textural (Giri*et al.* 2017) and sensory attributes.

Conclusion

This study aimed to enrich PCS with phytosterols, known for their cholesterol-lowering effects. The findings indicate that increasing phytosterols levels significantly ($p< 0.05$) altered physico-chemical, rheological, and sensory properties. At higher phytosterols levels (3% and 4%), the total sensory score significantly decreased ($p< 0.05$). Consequently, 2% phytosterols addition was optimized for PCS. However, phytosterols addition increased total saturated fatty acids while decreasing total unsaturated fatty acids. SEM analysis showed that insoluble phytosterols severely disrupted the cheese matrix.

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