Journal Pre-proof

Physicochemical and Textural Optimization of Plant-Based Cheese Using Lentil–Gluten Protein Systems

Elham Alehosseini, Paul L.H. McSweeney, Paul D. Cotter, Song Miao

PII: S0023-6438(26)00188-X

DOI: https://doi.org/10.1016/j.lwt.2026.119178

Reference: YFSTL 119178

To appear in: *LWT*

Received Date: 11 October 2025

Revised Date: 2 February 2026

Accepted Date: 18 February 2026

Please cite this article as: Alehosseini, E., McSweeney, P.L.H., Cotter, P.D., Miao, S., Physicochemical and Textural Optimization of Plant-Based Cheese Using Lentil–Gluten Protein Systems, *LWT*, https://doi.org/10.1016/j.lwt.2026.119178.

Physicochemical and Textural Optimization of Plant-Based Cheese Using Lentil–Gluten Protein Systems

Elham Alehosseini [a], Paul L.H. McSweeney [b], Paul D. Cotter [a], Song Miao [a*]

[a] Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, P61 C996, Ireland

[b] School of Food and Nutritional Sciences, University College Cork, T12 Y337, Ireland

*Corresponding author: Tel.: +353-(0)-25-42468, song.miao@teagasc.ie

CRediT authorship contribution statement

Elham Alehosseini: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing - Original Draft, Writing - Review & Editing

Paul L.H. McSweeney: Writing - Review & Editing.

Paul D. Cotter: Writing - Review & Editing.

Song Miao: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing - Review & Editing.

Funding

This project has received funding from the European Union's Horizon 2022 research and innovation programme under the Marie Sklodowska-Curie (MSCA-Postdoctoral Fellowships 2022) grant agreement No. 101105558 — NovelPBC — HORIZON-MSCA-2022-PF-01.

Physicochemical and Textural Optimization of Plant-Based Cheese Using Lentil–Gluten Protein Systems

Abstract

The rising demand for sustainable foods has accelerated interest in plant-based cheese; however, replacing casein with plant proteins often compromises texture, meltability, and overall quality. This study investigated lentil–gluten protein systems in dairy-free cheese formulations and evaluated the effects of protein, modified tapioca starch, chitosan, maltodextrin, and oil using response surface methodology (RSM). A central composite design with 50 formulations was used to model effects on moisture, protein, fat, ash, color, meltability, and texture properties. Functional characterization showed that modified tapioca starch exhibited the highest swelling capacity (9.21 mL/g) and water retention capacity (WRC) (10.03 g/g), while lentil protein showed the greatest water solubility index (WSI) (9.47%), and oil-holding capacity (OHC) (0.61 g/g). Linear models accurately described trends in moisture, ash, fat, and protein contents (R^2 = 0.95–0.99). Protein ($p < 0.01$), chitosan ($p < 0.05$), and starch ($p < 0.05$) significantly increased hardness, cohesiveness, and springiness, whereas oil enhanced meltability ($p < 0.01$). Higher protein levels decreased lightness (L^*) but increased a^* and b^* values, reflecting natural pigments and Maillard reaction products. The optimized formulation—28.31% protein powder, 20% oil, 1.5% chitosan, 9.99% starch, and 2.03% maltodextrin—achieved high overall desirability and closely matched predicted values, confirming model validity. Overall, this study provides the first integrated quantitative assessment of lentil–gluten systems in plant-based cheese and identifies ingredient interactions that govern structure and melting behavior. The findings establish a data-driven formulation framework capable of producing high-protein, texturally stable dairy-free cheese with improved functional and sensory potential.

Keywords: Plant-based cheese; Lentil protein; Gluten protein; Response surface methodology; Texture; Ingredient interactions

1. Introduction

A transition from meat and dairy to plant-based diets has been prioritized as a strategy to meet the nutritional demands of a growing global population while reducing greenhouse gas emissions and mitigating the environmental impact of animal-based food production, particularly cheese (Grasso, Roos, Crowley, Arendt, & O'Mahony, 2021). Conventional cheese, produced from milk, starter cultures, salt, and enzymes through fermentation, remains one of the most widely consumed and nutrient-rich foods. Its popularity continues to grow at ~3% annually, largely due to its high protein, fat, and vitamin content (D. Zhang et al., 2024). A number of plant-based food products—developed from ingredients such as nuts, grains, and legumes—have been designed to replicate the appearance, flavor, and texture of animal-based foods (Alehosseini, McSweeney, & Miao, 2025b; Boehm, Nicholson, & Baier, 2023). These alternatives can be rich in essential amino acids, phenolics, vitamins, minerals, and fiber, and may be consumed for health, environmental, ethical, and dietary reasons (McClements & Grossmann, 2023). The global plant-based food market is projected to reach $113 billion by 2031, positioning plant proteins from cereals, pulses, nuts, oilseeds, and pseudocereals as sustainable and cost-effective dairy replacements (Pereira et al., 2024; D. Zhang et al., 2024). However, many plant proteins exhibit limited solubility, restricting their suitability for cheese production (Wouters, Rombouts, Fierens, Brijs, & Delcour, 2016). Consumers may choose plant-based cheese due to lactose intolerance, milk allergy, or lifestyle choice, further driving innovation in this field (Aydar, Tutuncu, & Ozcelik, 2020).

Cheese analogues—products in which dairy proteins are partially or entirely substituted with plant-based ingredients—offer advantages such as shorter processing time, reduced cost, and nutritional flexibility (Kamath, Basak, & Gokhale, 2022). However, replacing casein with plant proteins often compromises texture and sensory quality, underscoring the need for a better understanding of ingredient functionality to improve dairy-free cheese formulations (Kamath et al., 2022; Masotti, Cattaneo, Stuknytė, & De Noni, 2018).

Among plant proteins, gluten and lentils hold particular promise for cheese applications (Naeem, Akhtar, Akram, Suleria, & Khalid, 2024; D. Zhang et al., 2024). Gluten, though largely insoluble in water, contains gliadins (ω5-, ω1,2-, α-, γ-) and glutenins, which provide desirable cheese-like characteristics including water and fat absorption, viscoelasticity, emulsification, and foaming (D. Zhang et al., 2024). Lentil protein—an underutilized source rich in essential amino acids—contains 11S and 7S globulins similar to those in soy and exhibits valuable functional properties (Tang, Roos, & Miao, 2024). Legumes in general are nutrient-dense, providing proteins, vitamins, and minerals, although they are limited in sulfur-containing amino acids. This limitation is often addressed by combining complementary proteins (Alehosseini, McSweeney, & Miao, 2025a; Kovačević, Bechtold, & Pham, 2024). Lentils, in particular, are low in saturated fat and calories while offering high-quality protein, making them both nutritionally and environmentally sustainable (Kussmann, Abe Cunha, & Berciano, 2023; Naeem et al., 2024).

In dairy-free cheese, protein gelation is critical to texture formation. Heat treatment induces denaturation, aggregation, and network formation, enabling plant globulins to behave similarly to dairy proteins. These processes are influenced by pH and protein composition, which determine hydrophobic interactions, disulfide bonding, and hydrogen bonding within the gel network (Ge et al., 2023; Nicolai, 2019; Tang et al., 2024).

Despite growing consumer demand, many plant-based cheeses remain nutritionally unbalanced, with lower protein and higher saturated fat contents than conventional cheese, increasing the risk of deficiencies in nutrients such as calcium and vitamin B2 (Taeger & Thiele, 2024).

To date, no published study has systematically investigated the combined application of lentil and gluten proteins within dairy-free cheese matrices, despite their complementary structural and functional attributes (Naeem et al., 2024; D. Zhang et al., 2024). Given that lentil globulins form heat-induced particulate networks with high water-binding capacity, while gluten proteins develop extensible viscoelastic structures through gliadin-mediated flow and glutenin-driven disulfide crosslinking, their co-assembly offers a mechanistically grounded strategy for improving matrix cohesion, hydration stability, and fat entrapment in plant-based cheese systems (Chompoorat, Fasasi, Lavine, & Rayas-Duarte, 2022; Jarpa-Parra, 2017).

In this study, a novel lentil (legume-derived)–gluten (cereal-derived) protein system was formulated to leverage the complementary gelation pathways, hydration dynamics, as well as nutritional and functional properties of these proteins to enhance the physicochemical and textural properties of plant-based cheese. The research first evaluated the functional characteristics of the individual proteins and starch, followed by systematic optimization of cheese formulations using response surface methodology (RSM) to model the interactive effects of protein, chitosan, starch, maltodextrin, and oil concentrations on product quality. The specific objectives were to: (i) assess the swelling capacity, solubility index, and oil-holding capacity (OHC) of key ingredients; (ii) formulate and optimize lentil–gluten cheese alternatives using RSM; (iii) characterize the optimized formulation in terms of composition, color, meltability, and texture; and (iv) elucidate ingredient interactions that govern structural and functional performance. This integrated approach provides mechanistic insights into ingredient interactions and establishes a framework for the rational design of nutritionally balanced, structurally robust, and sensory-appealing dairy-free cheese alternatives. The study's main strength lies in its innovative combination of complementary plant proteins and the application of RSM for data-driven formulation optimization. However, the work was conducted at laboratory scale and focused on instrumental analyses; storage stability was not evaluated. Future investigations should therefore include shelf-life assessments to substantiate the practical and commercial relevance of the developed formulations.

2. Materials and methods

2.1. Materials

The lentil protein powder (~81% protein) and modified tapioca starch were provided by HSN (Albolote, Spain) and 4mular (San Francisco, CA, US), respectively. Gluten from wheat (CAS Number: 8002-80-0, ≥ 75% protein), chitosan (low molecular weight, CAS Number: 9012-76-4, ≥ 75% deacetylated), and maltodextrin (CAS Number: 9050-36-6) were also supplied by Sigma-Aldrich. Moreover, coconut oil, Italian organic high oleic sunflower oil, acetic acid (CAS Number: 64-19-7, 99-100% purity), and salt were purchased from Coco loco (Naas, Ireland), Benvolio (Treviso, Italy), Lennox laboratory supplies ltd (Dublin, Ireland), and local supermarket (Fermoy, Ireland).

2.2. Functional analysis of plant proteins and starch

2.2.1. Swelling capacity

The swelling capacity of protein and starch samples was determined following Talens et al. (2022) with some modifications. Briefly, 0.2 g of each sample was placed in a graduated cylinder containing 10 mL of distilled water at 25 ℃. The sample was gently stirred to ensure uniform dispersion, then covered and left undisturbed at 25 ℃ for 18 h to allow full hydration and equilibration. After this period, the volume occupied by the swollen sample was recorded.

The swelling capacity was calculated using Equation 1 (Figure 1A). Measurements were performed in triplicate and mean values are reported.

$$\text{Swelling Capacity (mL/g)} = \frac{\text{Volume occupied by sample}}{\text{Original sample dry mass}} \quad (1)$$

INSERT FIGURE 1 ABOUT HERE

2.2.2. Water retention capacity (WRC)

The WRC of the samples was determined according to Robertson et al. (2000). Briefly, 3 g of sample was hydrated in 30 mL of distilled water at 25 °C and allowed to equilibrate for 18 h. Following hydration, the samples were centrifuged at 3,000 × g for 20 min (SCRVALL LYNX 6000, Thermo Scientific, Waltham, MA, USA). The supernatant was carefully decanted by inverting the tube, allowing the pellet to drain in place. The WRC was calculated using Equation 2 (Figure 1B). All measurements were performed in triplicate and mean values are reported.

$$\text{WRC } (^{g}/_{g}) = \frac{\text{Residue fresh mass} - \text{Residue dry mass}}{\text{Residue dry mass}} \quad (2)$$

2.2.3. Water solubility index (WSI) and oil-holding capacity (OHC)

To determine the WSI of the proteins (Equation 3), 2 g of each sample was dispersed in 100 mL of distilled water and heated in a water bath at 80 °C for 30 min. The mixture was then centrifuged at 1100 × g for 10 min at 25 °C. The supernatant was carefully collected and dried in an oven (FD 53, BINDER, Tuttlingen, Germany) at 103 ± 2 °C (Figure 1C) (Talens et al., 2022).

$$\text{WSI } (\%) = \frac{\text{Mass of dissolved solid in supernatant}}{\text{Mass of dry solids}} \times 100 \quad (3)$$

The OHC was determined according to Garcia-Fontanals, Llorente, Valderrama, Bravo, and Talens (2023) using Equation 4. Briefly, 1 g of the sample was mixed with 10 mL of sunflower oil using a vortex mixer (FINEVORTEX, FINEPCR Co., Ltd. Gunpo-si, South Korea) for 30 min, then centrifuged at 8000 × g for 20 min. After centrifugation, the supernatant was decanted and the remaining pellet was weighed (Figure 1D).

$$\text{OHC } \left({}^{g}/_{g}\right) = \frac{\text{Mass of sample after oil absorption} - \text{Dry sample mass}}{\text{Dry sample mass}} \quad (4)$$

All measurements were performed in triplicate and mean values are reported.

2.3. Formulation of dairy-free cheese alternatives

The dairy-free cheese alternatives were formulated according to the procedure of by Dobson and Marangoni (2023) with some modifications. All formulations corresponding to the 50 experimental runs of the central composite design were prepared under identical processing conditions. A 5% (w/w) protein solution was prepared by dissolving the plant-based protein blend (lentil protein 66.7 % and gluten 33.3 %) in the aqueous phase of deionized water (pH 5.1-5.4). The mixture was stirred at 400 rpm for 10 min using a magnetic stirrer (IKA® RET basic, IKA-Werke GmbH & Co. KG, Staufen im Breisgau, Germany) to ensure proper protein dispersion. The coconut oil was liquefied by heating to approximately 35-40 °C, mixed with high oleic sunflower oil at a ratio of 75:25, and incorporated into the protein solution under homogenization at 10,000 rpm for 5 min by an Ultra Turrax (IKA® T25, IKA-Werke GmbH &

Co. KG, Staufen im Breisgau, Germany). The emulsion was then combined with the remaining dry ingredients—protein, modified tapioca starch, chitosan, maltodextrin, and salt—and mixed until no visible clumps remained. The mixture was heated in a temperature-controlled water bath (Medical Supply Co. Ltd, Dublin, Ireland) at 80 °C for 3 min, followed by blending at low speed for 2 min in a laboratory blender (Waring Commercial, McConnellsburg, PA, USA). The heated mixture was maintained at 40 °C with gentle stirring for 10 min, poured into molds, and stored at 4 °C for 24 h prior to analysis. Across all trials, the mean formulation yield was 96.92 ± 2.86 %, calculated as the ratio of recovered solidified product mass to the initial formulation mass. Ingredient ranges used in the dairy-free cheese alternatives are summarized in Table 1.

Table 1. Ranges of Ingredients Used in Dairy-Free Cheese Formulations

Ingredients	**(%)**
Lentil protein (66.7%) + gluten (33.3%)	(20, 25, and 30)
Coconut oil (75%) + sunflower oil (25%)	(10, 15, and 20)
Chitosan	(0.5, 1, and 1.5)
Modified tapioca starch	(5, 7.5, and 10)
Maltodextrin	(2, 3, and 4)
Salt	1

2.4. Moisture, ash, fat, and protein content

The moisture and ash contents of dairy-free cheese alternatives were analyzed using a TGA-701 thermogravimetric analyzer (LECO Instruments, St. Joseph, MI, USA). The total protein of the samples was determined using an FP628 nitrogen analyzer (LECO, St. Joseph, MI, USA) with a nitrogen to protein conversion factor of 6.25. Additionally, the fat content was determined by SMART 6 + ORACLE systems (CEM Corporation, Matthews, NC, USA) (Fan et al., 2023; Tang, Roos, & Miao, 2023).

2.5. Color profile determination of dairy-free cheese alternatives

The colorimeter analysis of samples carried out by using a digital colorimeter (Chroma Meter CR-400, Konica Minolta Sensing, Osaka, Korea) based on the CIE $L^*a^*b^*$ system. The values of L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness) were determined. Additionally, the parameters ΔE, WI, and C^*_{ab} were calculated using Equations 5–7, respectively (A. Alehosseini et al., 2022; Garcia-Fontanals et al., 2023; Naeem et al., 2024).

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (5)$$

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (6)$$

$$C^*_{ab} = \sqrt{a^{*2} + b^{*2}} \quad (7)$$

2.6. Meltability

A cylindrical dairy-free cheese alternatives (14 mm in height and 25 mm in diameter) were stored in the refrigerator at 4 °C for 10 min, and then transferred to the oven (FD 53, BINDER, Tuttlingen, Germany) preheated to 232 °C for 5 min. After removal, the samples were allowed to cool for approximately 30 min before measuring the spread diameter at four different angles. A paper template with a 100 mm diameter was printed, featuring concentric circles at 5 mm intervals and lines at 45° angles, which were placed at the bottom of each glass plate, facing upwards. The meltability was calculated by determining the percentage increase in the average diameter from the initial diameter (Dobson & Marangoni, 2023).

2.7. Texture profile analysis (TPA)

The texture properties of dairy-free cheese alternatives were evaluated using a Texture Analyser TA.XT.Plus (Stable Micro Systems, Godalming, UK). After 24 h of storage at 4 °C, cylindrical samples (14 mm in height and 25 mm in diameter) were compressed at room temperature to 30% of their original height in a double compression with a SMS P/75R probe at a fixed speed of 1.0 mm/s. For each sample, textural parameters, including hardness, cohesiveness, springiness, adhesiveness, and resilience, were calculated by the software Texture Exponent (Version 6.2, Stable Micro Systems, Godalming, UK) (Grasso, Roos, Crowley, & O'Mahony, 2024; Monga, Dev, & Singhal, 2022).

2.8. Statistical analysis

For the functional properties of proteins and starch, all analyses were performed in triplicate. Mean comparisons were conducted using analysis of variance (ANOVA), with significance determined at $P < 0.05$. Post hoc multiple comparisons were assessed using Tukey's test in JMP® Pro software (Version 18.0.1, SAS Institute Inc, Cary, NC, USA). For dairy-free cheese alternatives, the experimental design and analysis were performed using central composite design (CCD) of RSM with 50 runs and 8 replications at the central point through ANOVA for a statistical significance $P < 0.05$, by using Design Expert software (Version 23, State-Ease, Minneapolis, MN, USA). In this study, five independent variables—the amounts of plant-based proteins, oil, chitosan, modified tapioca starch, and maltodextrin—were considered with three levels. The selected variable ranges were determined based on pre-tests and previous research. For each point, moisture, ash, fat, protein, color parameters, meltability, and texture properties of the dairy-free cheese alternatives were determined as responses. A second order polynomial equation was also applied to determine responses (Equation 8).

$$Y = a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4 + a_5X_5 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{44}X_4^2 + a_{55}X_5^2 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{14}X_1X_4 + a_{15}X_1X_5 + a_{23}X_2X_3 + a_{24}X_2X_4 + a_{25}X_2X_5 + a_{34}X_3X_4 + a_{35}X_3X_5 + a_{45}X_4X_5 + a_0 \quad (8)$$

Where a_0 is a constant and a_i, a_{ii}, and a_{ij} are also the linear, quadratic, and interactive coefficients, and X_1, X_2, X_3, X_4, and X_5 are the protein powder, oil, chitosan, starch, and maltodextrin concentrations. For formulation optimization using RSM, fat content and hardness were set to be maximized, with pure protein content fixed at approximately 25%, and all other responses constrained within acceptable ranges.

3. Results and discussion

3.1. Functional analysis of plant proteins and starch

Swelling capacity is strongly influenced by the structural characteristics and composition of biopolymers (Hoover, 2001). As shown in Table 2, the swelling capacities of modified tapioca starch, gluten, and lentil protein were 9.21, 5.32, and 4.83 mL/g, respectively. The superior swelling of modified starch likely results from structural alterations (e.g., cross-linking, substitution, or depolymerization) that disrupt the native granular organization and increase the exposure of hydrophilic hydroxyl groups. This enhances water penetration and retention within amorphous regions of starch granules, leading to pronounced swelling (BeMiller, 2018; Hoover, 2001). By contrast, proteins such as gluten and lentil protein possess compact tertiary and quaternary structures stabilized by hydrophobic interactions, disulfide bonds, and hydrogen bonding. These conformations restrict water accessibility, thereby limiting swelling. Gluten, composed mainly of glutenin and gliadin, forms an elastic, cohesive network upon hydration that resists expansion, while lentil protein, though slightly more soluble, remains a globular protein with constrained swelling compared to polysaccharides like starch (Damodaran, Parkin, & Fennema, 2007; Jarpa-Parra, 2017; Wieser, 2007).

Table 2. Swelling capacity, water retention capacity (WRC), water solubility index (WSI), and oil-holding capacity (OHC)

Sample	Swelling capacity (mL/g)	WRC (g/g)	WSI (%)	OHC (g/g)
Gluten	5.32[b] ± 0.31	1.50[c] ± 0.02	2.32[b] ± 0.41	0.54[b] ± 0.01
Lentil protein	4.83[b] ± 0.06	2.98[b] ± 0.11	9.47[a] ± 0.02	0.61[a] ± 0.06
Modified tapioca starch	9.21[a] ± 0.87	10.03[a] ± 0.03	–	–

These differences in swelling behavior have direct implications for cheese-matrix development. Modified starch, with its high swelling capacity, contributes to the formation of a continuous, hydrated phase capable of controlling serum separation and enhancing gel firmness—effects widely documented for physically or chemically modified starches used in processed cheese systems. In contrast, the modest swelling observed for lentil and gluten proteins suggests their role is less water immobilization and more network formation, where protein–protein contacts (non-covalent interactions for lentil globulins and disulfide-mediated elasticity for gluten) provide structural reinforcement rather than bulk hydration. This distinction highlights why combining starch with complementary proteins is mechanistically advantageous in plant-based cheese design: each biopolymer contributes unique functional attributes that together regulate water distribution, porosity, and viscoelasticity (Dobson, Laredo, & Marangoni, 2022; Jarpa-Parra, 2017; Trivedi et al., 2008; Wieser, 2007).

Lentil protein exhibited a significantly higher WRC than gluten, likely due to the presence of residual non-protein components in lentil protein, including dietary fibers and non-gluten polysaccharides, which provide additional hydrophilic binding sites. Moreover, the globular structure of lentil protein may expose polar amino acid residues during isolation, enhancing its ability to bind water (Boye, Zare, & Pletch, 2010; Karaca, Low, & Nickerson, 2011). The higher WRC of lentil protein can also be attributed to its greater content of charged amino acids and water-accessible side chains, which increase water binding compared to gluten. Lentil globulins undergo partial unfolding during extraction, exposing hydrophilic residues that increase water uptake and capillary-driven retention within the protein matrix (Jarpa-Parra et al., 2014). In contrast, gluten's polymeric glutenin network is stabilized by extensive intermolecular disulfide bonds that reduce segmental mobility and limit the formation of water-binding cavities (Wieser, 2007). These mechanistic differences explain the superior water-holding performance of lentil protein and indicate that lentil protein may help mitigate syneresis tendencies in dairy-free cheese formulations—an observation supported by previous work with legume-based gels (Boye, Aksay, et al., 2010; Lam, Can Karaca, Tyler, & Nickerson, 2018; Stone, Karalash, Tyler, Warkentin, & Nickerson, 2015).

The WSI, defined as the fraction of protein dissolved in water or buffer, was 2.32% for gluten and 9.47% for lentil protein (Table 2), indicating the latter's significantly greater solubility. Protein solubility depends on the balance between hydrophobic protein–protein and hydrophilic protein–solvent interactions and is affected by ionic strength, pH, temperature, and amino acid composition. Solubility is typically lowest near the isoelectric point (pI), where reduced electrostatic repulsion favors aggregation (Lam et al., 2018; Tang et al., 2023). Jarpa-Parra et al. (2014) reported solubility values of ~2–11% for lentil protein near pH 4–5, with higher solubility under acidic or alkaline conditions. Similarly, Hanley, Dobson, Stobbs, and Marangoni (2025) observed lentil protein isolate solubility of 3.7% at pH 5 and 6.2% at native pH. The higher WSI observed in this study may be attributed to the relatively high abundance of polar amino acids in lentil protein (e.g., glutamine, asparagine, arginine, lysine, glutamic acid, and aspartic acid), which enhance hydrogen bonding and electrostatic interactions with water (Jarpa-Parra et al., 2014). By contrast, gluten contains a higher proportion of hydrophobic residues, with many polar residues engaged in intra- and intermolecular bonding, which restricts solubility (Wieser, 2007). The solubility contrast is highly relevant to cheese structuring. Lentil protein's higher WSI suggests that it can disperse more uniformly during hydration and thermal treatment, forming finer particulate aggregates and contributing to a more homogeneous gel network. In contrast, gluten's low solubility and tendency to form cohesive, elastic strands support the development of larger-scale network continuity rather than fine particulate dispersion. This complementary behavior offers a mechanistic rationale for pairing these two proteins: lentil protein contributes water-distributed particulate domains, while gluten provides elasticity and network coherence—an interaction that has not been previously reported in plant-based cheese matrices (Boye, Aksay, et al., 2010; Dobson et al., 2022; Wieser, 2007).

OHC reflects the ability of proteins or biopolymers to physically entrap or bind oil and is primarily governed by hydrophobic interactions between nonpolar lipid chains and exposed hydrophobic amino acid side chains. Structural features such as porosity, surface area, and flexibility of the protein matrix, as well as oil properties (e.g., viscosity, polarity, and droplet size), also influence OHC (Kaur & Singh, 2007; Lam et al., 2018; Tang et al., 2023). In this study, lentil protein showed a slightly higher OHC (0.61 g/g) than gluten (0.54 g/g), likely due to greater surface hydrophobicity and a more open structural conformation, which facilitate lipid interactions (Boye, Zare, et al., 2010). These results are consistent with previous findings; for example, De Angelis et al. (2021) reported oil absorption values for red lentil flour between 0.32 and 0.45 g/g, depending on particle size and pre-treatment. The slightly higher OHC of lentil protein has important implications for fat immobilization in cheese analogues. Proteins with higher OHC can stabilize dispersed oil droplets more effectively during emulsification and heating, reducing free-oil formation and enhancing meltability—critical attributes in cheese analogues (McClements, 2015). The OHC values observed in this study exceed those reported for native lentil flour, likely due to differences in protein purity, particle size, and processing conditions during isolate production (Boye, Zare, et al., 2010; Klupšaitė & Juodeikienė, 2015).

Together, the WRC, WSI, and OHC data demonstrate that lentil protein presents a multifunctional profile (high hydration + moderate solubility + moderate OHC), while gluten provides network elasticity—highlighting the scientific rationale for combining these two proteins in dairy-free cheese systems.

3.2. Moisture, ash, fat, and protein content

The moisture content of the plant-based cheese formulations ranged from 29.68% to 60.20%. A significant negative linear relationship was observed between moisture content and the levels of protein, oil, modified tapioca starch, and maltodextrin (Equation 9), indicating that increasing the proportion of these ingredients reduced the amount of free water within the matrix. This trend reflects both hydrophilic water-binding and hydrophobic water-excluding effects. Lentil protein and gluten exhibit moderate to high water-binding capacity due to their polar amino acid side chains and partially unfolded structures; however, when incorporated at higher levels, they increase total solids and consequently decrease the proportion of free water (Boye, Zare, et al., 2010; McClements, 2015). Similarly, lipids—particularly coconut and high-oleic sunflower oils—are hydrophobic and displace water within the emulsion, limiting overall moisture retention (McClements, 2015). Modified tapioca starch and maltodextrin also reduce moisture by binding water through hydrogen bonding and forming viscous or gel-like structures that immobilize water molecules (BeMiller, 2018). The pronounced negative effect of both ingredients suggests competitive water immobilization among matrix components. Modified starch granules swell extensively during heating, reducing the availability of free water, while maltodextrin lowers water activity by forming hydrogen-bonded water clusters that contribute to a denser matrix microstructure. Such competition for water is characteristic of starch−protein systems and explains the sharper moisture decline observed at higher inclusion levels (Hoobin et al., 2013; Liu, Xie, Yu, Chen, & Li, 2009).

As expected, increasing oil and plant protein levels directly elevated fat and protein contents, respectively, reflecting their compositional contributions, as predicated by Equations 11 and

12 (Alehosseini et al., 2025a). The protein content of the formulations ranged from 17.67% to 27.63%, while the ash content ranged from 1.02% to 2.98%, reflecting the intrinsic mineral composition of the raw materials. Lentil protein, rich in potassium, phosphorus, and iron, was the primary contributor to ash, while the gluten powder likely contributed only trace minerals, originating from residual salts or processing residues (Iqbal, Khalil, Ateeq, & Sayyar Khan, 2006). Chitosan, a deacetylated derivative of chitin, may also contribute trace minerals such as calcium, magnesium, and phosphorus, thereby elevating ash levels even at low concentrations (Younes & Rinaudo, 2015). In contrast, refined lipids (i.e., coconut and sunflower oils) are essentially mineral-free, and carbohydrate-based ingredients such as maltodextrin and tapioca starch contain negligible ash due to their high purification levels (BeMiller, 2018; Iqbal et al., 2006; Stevens, 2020). The relatively narrow variation in ash compared with moisture and protein suggests that mineral-bearing ingredients played a secondary but consistent structural role. This is consistent with observations in other legume-based systems, where minerals contribute to ionic bridging and mild protein aggregation, subtly affecting firmness and water mobility rather than significantly altering total ash content (Shand, Ya, Pietrasik, & Wanasundara, 2007; Stone et al., 2015). These observations align with previous studies. Grasso et al. (2024) reported moisture levels of 55.4–56.6% in plant-based cheese systems containing zein, chickpea protein concentrate, and tapioca starch. Similarly, Ndatsu et al. (2023) observed protein levels of 31.2–39.2%, moisture contents of 42.3–54.3%, fat levels of 13.4–23.5%, and ash contents of 4.4–6.4% in tofu produced from soybeans and Bambara groundnut. By contrast, lentil milk–based soft cheeses typically exhibit much higher moisture (72.44–79.58%) and lower protein contents (3.16–6.74%), reflecting differences in protein concentration, matrix structure, and processing methods (Naeem et al., 2024). Compared to previous plant-based cheese studies, the present formulations exhibit noticeably higher protein content. Gluten forms a viscoelastic network that can expel free water during heating, while lentil globulins undergo

partial aggregation and exhibit limited swelling capacity (Cornet, van der Goot, & van der Sman, 2020; Hong, Wanasundara, Nickerson, & Shand, 2012). Together, these behaviors produce a more compact, protein-dense matrix, consistent with the observed reductions in moisture and the higher protein concentration. This mechanistic interaction distinguishes the lentil–gluten system from plant cheeses based on single-protein sources, where either water retention or network formation dominates.

ANOVA results confirmed that the linear models accurately captured the experimental trends in composition, with coefficients of determination (R^2) of 0.95 (moisture), 0.95 (ash), 0.99 (fat), and 0.97 (protein) (Table 3). Most formulation variables—including protein, oil, modified starch, and maltodextrin—had significant effects on moisture ($p < 0.01$). The high R^2 values indicate strong model predictability, with moisture, fat, protein, and ash responding as expected to their primary ingredients. Moisture exhibited multicomponent competition, reflecting the complex interactions among proteins, starches, and polysaccharides, whereas fat and ash behaved largely additively.

393 **Table 3**. Analysis of variance (ANOVA) for moisture, ash, fat, and protein content

	Moisture content		Ash		Fat content		Protein content	
Source	**Sum of Squares**	**p-value**	**Sum of Squares**	**p-value**	**Sum of Squares**	**p-value**	**Sum of Squares**	**p-value**
Model	2038.36	< 0.0001	18.17	< 0.0001	843.41	< 0.0001	581.13	< 0.0001
Protein (X_1)	767.12	< 0.0001	17.87	< 0.0001	0.0467	0.4800	580.18	< 0.0001
Oil (X_2)	986.26	< 0.0001	0.0482	0.1415	842.91	< 0.0001	0.0241	0.7924
Chitosan (X_3)	0.3380	0.7131	0.2423	0.0016	0.2406	0.1131	0.2119	0.4370
Modified tapioca starch (X_4)	217.12	< 0.0001	0.0036	0.6842	0.1943	0.1534	0.6533	0.1754
Maltodextrin (X_5)	67.51	< 0.0001	0.0068	0.5773	0.0170	0.6696	0.0568	0.6866
Residual	108.59		0.9459		4.05		15.15	
Lack of Fit	97.92	0.2294	0.9217	0.0605	3.65	0.2274	14.28	0.0612
Pure Error	10.67		0.0242		0.3964		0.8722	
Cor Total	2146.96		19.12		847.46		596.28	

394

To provide a more quantitative description of these effects, predictive regression models were developed (Equations 9-12). These models quantify how protein powder (X_1), oil (X_2), chitosan (X_3), modified tapioca starch (X_4), and maltodextrin (X_5) influence the compositional attributes of the formulations. The sign and magnitude of each coefficient indicate the direction and strength of each ingredient's effect. For example, negative coefficients in the moisture model highlight water displacement or immobilization by solids, whereas positive coefficients in the ash and protein models reflect compositional enrichment from mineral- and protein-rich ingredients. The fat model, meanwhile, shows a nearly one-to-one increase in fat content with oil addition, consistent with its direct contribution to the formulation. Collectively, these equations confirm and extend the experimental observations, while offering predictive capability for future formulation optimization.

$$\text{Moisture (\%)} = -\,0.95\,X_1 - 1.08\,X_2 - 1.01\,X_4 - 1.41\,X_5 + 97.32 \quad (9)$$

$$\text{Ash (\%)} = 0.14\,X_1 + 0.17\,X_3 - 1.74 \quad (10)$$

$$\text{Fat (\%)} = 0.99\,X_2 + 0.55 \quad (11)$$

$$\text{Protein (\%)} = 0.83\,X_1 + 1.51 \quad (12)$$

3.3. Color profile determination of dairy-free cheese alternatives

The color of dairy-free cheese alternatives is influenced by intrinsic factors (e.g., ingredient type and concentration, fat content, and functional additives) and extrinsic factors (e.g., processing, packaging, and storage), all of which influence visual appeal and consumer acceptance (Bekele, Hansen, Eshetu, Ipsen, & Hailu, 2019).

In this study, L^*, a^*, and b^* values ranged from 58.28 to 83.32, –0.06 to 1.11, and 16.04 to 23.97, respectively, indicating noticeable variability in brightness and chromaticity due to formulation differences. ANOVA results (Table 4) showed that linear models provided excellent fits for L^*, a^*, ΔE, and WI, with R^2 values of 0.91, 0.83, 0.91, and 0.93, respectively. In contrast, two-factor interaction (2FI) models better explained variation in b^* and C^*_{ab}, R^2 values of 0.96 for both. These results emphasize the importance of ingredient interactions on color attributes. Protein ($p < 0.01$), oil ($p < 0.01$), and modified starch ($p < 0.05$) significantly affected L^*, b^*, ΔE, WI, and C^*_{ab}. Chitosan had a significant effect on L^*, ΔE, and WI ($p < 0.01$), while protein and modified starch strongly influenced a^* ($p < 0.01$). Maltodextrin also significantly affected L^*, b^*, WI, and C^*_{ab} ($p < 0.05$). Among interactions, protein–oil (X_1X_2) had a pronounced effect on b^* and C^*_{ab} ($p < 0.01$).

Table 4. Analysis of variance (ANOVA) for $L^*, a^*, b^*, \Delta E, WI$, and C^*_{ab}

	L^*		a^*		b^*		ΔE		WI		C^*_{ab}	
Source	**Sum of Squares**	**p-value**	**Sum of Squares**	**p-value**	**Sum of Squares**	**p-value**	**Sum of Squares**	**p-value**	**Sum of Squares**	**p-value**	**Sum of Squares**	**p-value**
Model	1187.71	< 0.0001	3.87	< 0.0001	174.14	< 0.0001	944.52	< 0.0001	970.51	< 0.0001	175.24	< 0.0001
Protein (X_1)	421.41	< 0.0001	3.62	< 0.0001	139.83	< 0.0001	218.44	< 0.0001	542.80	< 0.0001	140.96	< 0.0001
Oil (X_2)	568.26	< 0.0001	0.0157	0.3507	25.91	< 0.0001	571.95	< 0.0001	280.80	< 0.0001	25.84	< 0.0001
Chitosan (X_3)	127.92	< 0.0001	0.0013	0.7874	0.3050	0.2288	104.30	< 0.0001	89.33	< 0.0001	0.3068	0.2282
Modified tapioca starch (X_4)	56.86	< 0.0001	0.2240	0.0009	0.9456	0.0381	41.89	< 0.0001	44.92	< 0.0001	0.9256	0.0404
Maltodextrin (X_5)	13.24	0.0271	0.0003	0.8978	1.25	0.0182	7.94	0.0626	12.66	0.0080	1.25	0.0184
Protein × Oil ($X_1 \times X_2$)					3.68	0.0002					3.71	0.0002
Protein × Chitosan ($X_1 \times X_3$)					0.3983	0.1704					0.4028	0.1689
Protein × Modified tapioca starch ($X_1 \times X_4$)					0.2869	0.2428					0.2945	0.2376
Protein × Maltodextrin ($X_1 \times X_5$)					0.3383	0.2055					0.3383	0.2063
Oil × Chitosan ($X_2 \times X_3$)					0.0520	0.6161					0.0553	0.6059
Oil × Modified tapioca starch ($X_2 \times X_4$)					0.0259	0.7233					0.0226	0.7413
Oil × Maltodextrin ($X_2 \times X_5$)					0.1263	0.4358					0.1288	0.4322

Chitosan × Modified tapioca starch ($X_3 \times X_4$)					0.0332	0.6887					0.0358	0.6779
Chitosan × Maltodextrin ($X_3 \times X_5$)					0.8033	0.0548					0.8033	0.0552
Modified tapioca starch × Maltodextrin ($X_4 \times X_5$)					0.1639	0.3753					0.1668	0.3721
Residual	111.40		0.7750		6.90		95.70		72.06		6.93	
Lack of Fit	111.01	0.0600	0.7102	0.1588	6.82	0.0752	95.40	0.0701	71.70	0.0619	6.85	0.0899
Pure Error	0.3868		0.0648		0.0800		0.3042		0.3568		0.0800	
Cor Total	1299.11		4.64		181.05		1040.22		1042.57		182.17	

Predictive models describing the effects of formulation variables on color attributes are presented in Equations (13–18). For example, negative coefficients in L^* indicate factors that decrease brightness, while positive coefficients in a^* or b^* highlight contributions to red or yellow hues, respectively—consistent with how color parameters respond to ingredient proportions in mixture design models of cheese analogue systems (Garcia-Fontanals et al., 2023).

$$L^* = -\,0.70\,X_1 - \,0.82\,X_2 - \,3.88\,X_3 + \,0.52\,X_4 + 0.62\,X_5 + 97.15 \quad (13)$$

$$a^* = +\,0.06\,X_1 + \,0.03\,X_4 - 1.45 \quad (14)$$

$$b^* = +\,0.04\,X_1 - 0.44\,X_2 - \,0.28\,X_4 - 1.05\,X_5 + 0.01\,X_1X_2 + 23.43 \quad (15)$$

$$\Delta E = +\,0.51\,X_1 + 0.82\,X_2 \, + 3.50\,X_3 - 0.44\,X_4 \, + 4.39 \quad (16)$$

$$WI = -0.80\,X_1 - 0.57\,X_2 - 3.24\,X_3 + \,0.46\,X_4 + \,0.61\,X_5 + 89.36 \quad (17)$$

$$C^*_{ab} = +0.04\,X_1 - 0.44\,X_2 - 0.28\,X_4 \, - 1.05\,X_5 + 0.01\,X_1X_2 + 23.44 \quad (18)$$

These results are consistent with previous findings. Naeem et al. (2024) reported L^*, a^*, and b^* values of 39.61–41.64, 0.13–7.97, and 11.43–15.01, respectively, in lentil milk–based soft cheese analogs, while Garcia-Fontanals et al. (2023) reported L^* = 62.57, a^* = –0.25, and b^* = 17.23 in hybrid faba bean–insect protein cheeses. Differences in protein type, pigment composition, and fat content likely explain the observed variability across studies (Chudy, Bilska, Kowalski, & Teichert, 2020; Sharan et al., 2021).

As confirmed by the predictive models (Equations 13–15), in the present study, increasing protein powder (lentil and gluten) decreased L^* while increasing a^* and b^*, reflecting contributions from natural pigments and heat-induced browning reactions, including Maillard reaction products formed during thermal processing (Martins, Jongen, & van Boekel, 2000; Sharan et al., 2021). Conversely, modified starch and maltodextrin, being white and highly dispersible, increased L^* while reducing b^* by diluting pigment dispersion and enhancing light scattering. Modified starch also increased a^*, likely by improving pigment dispersion within the matrix. Oils reduced L^* and b^*, possibly by diminishing light scattering and diluting water-soluble pigments. Variations in b^* may also reflect differences in carotenoid levels (e.g., β-carotene, lutein, zeaxanthin) inherent to protein and oil components (Chong & Wong, 2017; Chudy et al., 2020; Naeem et al., 2024; Wannasin & McClements, 2023). Overall, these results not only quantify color changes but also provide causal insights into ingredient interactions and pigment distribution, offering guidance for designing visually appealing dairy-free cheeses.

3.4. Meltability

Meltability, a key functional property of cheese, describes the extent and uniformity of softening and flow upon heating. In plant-based cheese, meltability is influenced by ingredient composition (e.g., type and ratio of proteins, fats, polysaccharides, and additives), as well as processing conditions, which together shape the microstructure, water mobility, and fat dispersion (Alehosseini et al., 2025b; Grasso et al., 2021; Rodríguez, 2017).

The meltability of the dairy-free cheese alternatives ranged from 1.20% to 24%, consistent with values reported for commercial plant-based cheeses (5.59–21.00%) by Grasso et al. (2021), but substantially lower than dairy cheeses, which exhibit greater meltability due to their casein matrix and emulsified fat structure (Kovačević et al., 2024). This difference highlights the limited ability of plant proteins to form heat-sensitive networks, in contrast to casein, which readily rearranges upon heating to enable flow, as demonstrated by rheological and microstructural studies of dairy cheese systems (Fox, Guinee, Cogan, & McSweeney, 2017). ANOVA results (Table 5) showed that meltability was best described by a quadratic model (R^2 = 0.93), indicating strong nonlinear effects of formulation variables on this property.

Table 5. Analysis of variance (ANOVA) for meltability, hardness, cohesiveness, springiness, and adhesiveness

	Meltability		Hardness		Cohesiveness		Springiness		Adhesiveness		Resilience	
Source	**Sum of Squares**	**p-value**	**Sum of Squares**	**p-value**	**Sum of Squares**	**p-value**	**Sum of Squares**	**p-value**	**Sum of Squares**	**p-value**	**Sum of Squares**	**p-value**
Model	1450.83	< 0.0001	5857.31	< 0.0001	0.3434	< 0.0001	12686.16	< 0.0001	61.55	< 0.0001	1047.60	< 0.0001
Protein (X_1)	266.56	< 0.0001	3560.10	< 0.0001	0.1725	< 0.0001	12229.54	< 0.0001	28.25	< 0.0001	409.16	< 0.0001
Oil (X_2)	569.90	< 0.0001	1202.34	< 0.0001	0.1274	< 0.0001	178.52	0.0015	3.34	0.0015	525.49	< 0.0001
Chitosan (X_3)	215.51	< 0.0001	580.32	< 0.0001	0.0122	0.0150	197.26	0.0009	5.97	< 0.0001	23.51	0.0089
Modified tapioca starch (X_4)	15.83	0.0448	472.34	< 0.0001	0.0246	0.0008	64.72	0.0482	0.1109	0.5269	64.81	< 0.0001
Maltodextrin (X_5)	5.12	0.2424	42.20	0.0865	0.0068	0.0648	16.11	0.3161	2.81	0.0031	24.62	0.0075
Protein × Oil ($X_1 \times X_2$)	41.40	0.0020							3.22	0.0017		
Protein × Chitosan ($X_1 \times X_3$)	0.0450	0.9117							6.73	< 0.0001		
Protein × Modified tapioca starch ($X_1 \times X_4$)	0.0050	0.9705							0.0490	0.6736		
Protein × Maltodextrin ($X_1 \times X_5$)	0.4050	0.7397							2.16	0.0085		
Oil × Chitosan ($X_2 \times X_3$)	99.40	< 0.0001							0.1867	0.4129		
Oil × Modified tapioca starch ($X_2 \times X_4$)	1.81	0.4845							0.5248	0.1742		
Oil × Maltodextrin ($X_2 \times X_5$)	2.20	0.4401							0.0655	0.6263		

Chitosan × Modified tapioca starch ($X_3 \times X_4$)	8.40	0.1373							0.0300	0.7414		
Chitosan × Maltodextrin ($X_3 \times X_5$)	9.25	0.1198							1.81	0.0150		
Modified tapioca starch × Maltodextrin ($X_4 \times X_5$)	0.6050	0.6848							0.0113	0.8398		
Protein^2 $(X_1)^2$	14.15	0.0569							0.0596	0.6422		
Oil^2 $(X_2)^2$	6.39	0.1929							0.2898	0.3092		
Chitosan^2 $(X_3)^2$	6.27	0.1972							0.0115	0.8379		
Modified tapioca starch^2 $(X_4)^2$	9.82	0.1094							0.0034	0.9111		
Maltodextrin^2 $(X_5)^2$	0.0912	0.8746							0.1978	0.3995		
Residual	104.37		604.17		0.0834		689.48		7.84		137.90	
Lack of Fit	104.07	0.1022	603.79	0.0650	0.0831	0.0609	665.12	0.1500	7.83	0.0600	137.38	0.0670
Pure Error	0.3000		0.3771		0.0003		24.36		0.0169		0.5194	
Cor Total	1555.20		6461.47		0.4269		13375.64		69.40		1185.49	

Protein, oil, and chitosan (p < 0.01), along with modified starch (p < 0.05), significantly influenced meltability. Notably, interactions between protein and oil (X_1X_2) and oil and chitosan (X_2X_3) were also significant (p < 0.01). The significant interaction terms reflect the complex network formation and plasticization mechanisms in plant-based cheese matrices (Lyu, Sala, & Scholten, 2023). To quantitatively assess the effects of formulation variables on meltability, a predictive regression model was developed, with coefficients indicating the direction and relative strength of each ingredient's influence (Equation 19):

$$\text{Meltability (\%)} = -2.02\,X_1 + 4.29\,X_2 - 13.87\,X_3 - 5.89\,X_4 - 0.04\,X_1X_2 - 0.70\,X_2X_3 + 100.44 \quad (19)$$

Protein had a strong negative effect on meltability. Higher levels of lentil protein and gluten likely formed dense, thermally stable networks reinforced by hydrogen bonds and disulfide bridges, limiting flow upon heating. Heat-induced Maillard cross-linking further reduced thermal flow (Grasso et al., 2022; Mattice & Marangoni, 2020; Sharan et al., 2021). Chitosan also decreased meltability, probably by forming electrostatic interactions with negatively charged proteins and starch phosphates, thereby reinforcing the gel network and restricting protein and fat mobility (Alehosseini, Shahiri Tabarestani, Kharazmi, & Jafari, 2022). Modified tapioca starch had a similar negative impact: upon heating, starch granules gelatinized and retrograded, forming semi-crystalline networks resistant to deformation (Hanley et al., 2025; Sutter, Assad-Bustillos, & Windhab, 2023; Ye, Hewitt, & Taylor, 2009). This effect explains why formulations with higher starch content exhibited lower meltability despite adequate oil content, as the crystalline starch domains act as physical barriers to flow. In contrast, oil content significantly enhanced meltability. Fats such as coconut oil and high-oleic sunflower oil acted as plasticizers, reducing matrix rigidity and lowering the melting point by interrupting protein–starch interactions (McClements & Grossmann, 2023; Sivakami, 2021). These data confirm prior findings that fat phase continuity facilitates melt flow in plant-based matrices (Dobson & Marangoni, 2023; Sanders, Dobson, & Marangoni, 2024). Processing conditions further influenced meltability: protein hydration prior to starch addition allowed partial unfolding and early network formation, controlled heating at 80 °C minimized excessive denaturation, and high shear mixing promoted starch swelling and fat dispersion. Nonetheless, high starch and protein concentrations still restricted meltability in certain formulations, consistent with prior observations (Dobson & Marangoni, 2023). These results suggest that meltability is not solely determined by individual ingredients but also by their interactions and processing-induced microstructural arrangements, emphasizing the importance of integrated formulation–processing strategies.

3.5. Texture profile analysis (TPA)

TPA is a widely used method for evaluating the mechanical and sensory properties of food, simulating the first two bites of mastication by compressing samples to a defined deformation level (Dobson & Marangoni, 2023). In this study, hardness, cohesiveness, springiness, adhesiveness, and resilience were measured to characterize the textural properties of the dairy-free cheese alternatives. ANOVA results (Table 5) and the predictive model (Equation 20) indicated that hardness increased significantly with higher levels of plant protein, oil, chitosan, and modified tapioca starch, while maltodextrin had no significant effect. This suggests that network formation and structural reinforcement primarily govern firmness, rather than simple water-binding effects (Dobson & Marangoni, 2025). Lentil protein (rich in globulins) and gluten (gliadin and glutenin) synergistically formed a viscoelastic network via protein–protein interactions, reinforced by thermal denaturation during processing (Gasparre, van den Berg, Oosterlinck, & Sein, 2022; Jo, Huang, & Chen, 2020). Coconut oil, owing to its high saturated fat content and sharp melting point, enhanced firmness through crystalline fat structuring upon cooling, while high-oleic sunflower oil contributed minimal softening. This aligns with prior findings that saturated fats can act as active structuring agents, whereas unsaturated fats may act primarily as lubricants, highlighting formulation-dependent fat–protein interactions (Sanders et al., 2024). Chitosan, a cationic biopolymer, likely increased hardness by forming electrostatic protein–polysaccharide interactions (Xie et al., 2024). Modified tapioca starch gelatinized during heating and retrograded upon cooling, providing rigidity and acting as a filler in the protein–polysaccharide matrix (N.-N. Zhang et al., 2022). The combined effect of starch and protein emphasizes the importance of non-covalent interactions and filler reinforcement in plant-based cheese matrices (Lyu, Sala, & Scholten, 2022). Comparable trends have been reported by Dobson and Marangoni (2023), although other systems (e.g., soy, pea) have shown the opposite effect, underscoring the formulation-dependent and ingredient-specific nature of textural outcomes (Mefleh et al., 2022; Rinaldoni, Palatnik, Zaritzky, & Campderrós, 2014).

Cohesiveness, which reflects the strength of internal bonding (Grasso et al., 2022), increased with protein, chitosan, and starch, but decreased with oil addition (Equation 21). Gluten contributed elasticity, lentil protein increased structural density, and chitosan strengthened the network via electrostatic and hydrogen bonding, while starch gelation and water-binding further supported cohesiveness (Boeck, Zannini, Sahin, Bez, & Arendt, 2021; N.-N. Zhang et al., 2022). In contrast, higher oil levels disrupted protein–polysaccharide continuity, leading to dispersed fat domains, consistent with previous observations in low-fat cheese analogues (Lim, Easa, Karim, Bhat, & Liong, 2011). This provides mechanistic insight into why cohesiveness decreases despite the presence of network-forming components.

Springiness followed the same trend: it was enhanced by protein, chitosan, and starch but reduced by oil (Equation 22). Elastic recovery was attributed to the viscoelastic protein network, further reinforced by chitosan gelation and starch retrogradation (Grasso et al., 2022; Mattice & Marangoni, 2020). Oils, particularly coconut oil, acted as plasticizers, weakening intermolecular linkages and reducing elastic recovery (Bhasney, Patwa, Kumar, & Katiyar, 2017; Dobson et al., 2022; Mattice & Marangoni, 2020).

Based on significant main effects in the fitted model (Equation 23), adhesiveness decreased with higher protein and chitosan but increased with oil and maltodextrin. A dense protein network, particularly with gluten cross-linking, limited water mobility and reduced stickiness (Ushkalova, Zhao, Gu, Wang, & Zhang, 2025). Chitosan further decreased adhesiveness by stabilizing gel structures (Xie et al., 2024). In contrast, oils reduced friction at the probe–sample interface, while maltodextrin's water-binding enhanced lubrication, producing a stickier texture (Akshit, Poswal, Kaushik, Deshwal, & Huppertz, 2025).

Resilience, the ability of the matrix to recover after deformation, was enhanced by protein, chitosan, and starch, but reduced by oil and maltodextrin (Equation 24). Protein–chitosan interactions promoted elasticity, while starch retrogradation strengthened recovery capacity. Conversely, oil disrupted network integrity, and maltodextrin diluted the protein phase, lowering resilience (Scott & Awika, 2023; Xie et al., 2024). This observation is novel in dairy-free cheese alternatives and suggests that resilience can be tailored by optimizing polysaccharide–protein ratios, an aspect not fully explored in previous plant-based cheese studies.

Collectively, these results indicate that desirable cheese-like textures in plant-based products rely on carefully balancing moisture retention, phase continuity, and non-covalent interactions among proteins, polysaccharides, and fats (Mattice & Marangoni, 2020; Naeem et al., 2024). This study highlights the unique role of chitosan as both a hardness and cohesiveness enhancer while modulating adhesiveness and resilience, an aspect that could inform future formulation strategies for dairy-free cheeses.

ANOVA results (Table 5) confirmed that hardness, cohesiveness, springiness, and resilience were best described by linear models (R^2 = 0.91, 0.80, 0.95, and 0.88, respectively), while adhesiveness fit a quadratic model (R^2 = 0.89). Plant protein ($p < 0.01$), oil ($p < 0.01$), and chitosan ($p < 0.05$) significantly influenced all TPA parameters. Modified tapioca starch significantly affected hardness ($p < 0.01$), cohesiveness ($p < 0.01$), springiness ($p < 0.05$), and resilience ($p < 0.01$), while maltodextrin significantly influenced adhesiveness and resilience ($p < 0.01$). Several interactions, including protein × oil, protein × chitosan, protein × maltodextrin, and chitosan × maltodextrin, also significantly shaped adhesiveness. These interactions indicate synergistic and antagonistic effects between ingredients, providing mechanistic understanding beyond simple main effects, which supports predictive formulation modeling. Predictive models were developed, with coefficients indicating the relative effect of protein, oil, chitosan, starch, and maltodextrin on hardness, cohesiveness, springiness, adhesiveness, and resilience (Equations 20–24).

$$\text{Hardness (N)} = 2.05\,X_1 + 1.19\,X_2 + 8.26\,X_3 + 1.49\,X_4 - 65.99 \quad (20)$$

$$\text{Cohesiveness} = 0.01\,X_1 - 0.01\,X_2 + 0.04\,X_3 + 0.01\,X_4 + 0.21 \quad (21)$$

$$\text{Springiness (\%)} = +3.79\,X_1 - 0.46\,X_2 + 4.82\,X_3 + 0.55\,X_4 - 30.09 \quad (22)$$

$$\text{Adhesiveness (N.s)} = -0.31\,X_1 + 0.05\,X_2 - 3.90\,X_3 + 0.17\,X_5 - 0.01\,X_1X_2 + 0.18\,X_1X_3 - 0.05\,X_1X_5 - 0.47\,X_3X_5 + 16.29 \quad (23)$$

$$\text{Resilience (\%)} = +0.69\,X_1 - 0.79\,X_2 + 1.66\,X_3 + 0.55\,X_4 - 0.85\,X_5 + 12.05 \quad (24)$$

3.6. Formulation optimization of dairy-free cheese alternatives

Optimization analysis showed that maximizing fat content and hardness, with protein content fixed at 25% and all other parameters constrained within acceptable ranges, yielded an optimal formulation consisting of 28.31% protein powder, 20% oil, 1.5% chitosan, 9.99% modified tapioca starch, and 2.03% maltodextrin. This combination produced a dairy-free cheese alternative with a high overall desirability (Figure 2).

INSERT FIGURE 2 ABOUT HERE

When compared with existing literature, the optimized formulation exhibits higher protein content than many commercial dairy-free cheeses (e.g., plant-based cheeses with 0.1-3% protein) while maintaining desirable textural properties, highlighting the innovation of using a combination of protein powder, chitosan, and modified starch to balance texture and nutritional value (Ali, O'Mahony, O'Sullivan, & Kerry, 2025; Grasso et al., 2021). These findings not only provide a scientifically grounded formulation strategy but also offer mechanistic insights into how the interplay of protein, fat, and polysaccharides governs the functional properties of dairy-free cheese alternatives, as evidenced by the significant main and interaction effects identified in the RSM models.

Validation of the RSM was conducted by preparing three replicates of the predicted optimal formulation and measuring their properties. The agreement between the experimental data and the predicted values also confirmed the predictability of the RSM model resulting from the optimal formulation (Table 6).

608 **Table 6.** Experimental validation data of the optimal formulation

	Experimental	Predicted		Experimental	Predicted		Experimental	Predicted
Moisture (%)	36.22	35.87	b^*	17.00	16.50	Cohesiveness	0.50	0.45
Ash (%)	2.59	2.48	ΔE	36.28	36.08	Springiness (%)	79.50	80.73
Fat (%)	20.58	20.35	WI	57.98	56.29	Adhesiveness (N.s)	4.23	4.69
Protein (%)	24.77	25.00	C^*_{ab}	17.01	16.51	Resilience (%)	22.24	22.04
L^*	61.58	61.57	Meltability (%)	6.00	5.76			
a^*	0.63	0.55	Hardness (N)	43.72	43.12			

609

4. Conclusion

This study provides a mechanistic and quantitative assessment of how lentil–gluten protein systems, in combination with modified tapioca starch, chitosan, maltodextrin, and blended vegetable oils, determine the composition, meltability, color, and texture of dairy-free cheese alternatives. Functional characterization revealed distinct and complementary ingredient behaviors: modified tapioca starch exhibited the highest swelling capacity (9.21 mL/g) and WRC (10.03 g/g), forming the primary hydration matrix, while lentil protein demonstrated markedly higher WSI (9.47%) and OHC (0.61 g/g) compared with gluten, supporting emulsification and uniform protein dispersion. The systematic application of RSM enabled precise modeling of both independent and interactive effects among formulation variables. Increasing protein from 20% to 30% increased hardness and cohesiveness, but reduced meltability due to the formation of thermally stable lentil–gluten networks reinforced by hydrogen bonding and disulfide cross-linking. Oil content, in contrast, improved meltability by plasticizing the matrix and disrupting protein–starch interactions, while starch and chitosan significantly enhanced hardness, cohesiveness, springiness, and resilience through gelatinization, retrogradation, and electrostatic interactions. Color parameters were strongly formulation-dependent, with increasing protein levels decreasing L^* but increasing a^* and b^*, an effect attributed to the concentration of lentil-derived carotenoids and phenolic pigments, as well as the formation of Maillard reaction chromophores during thermal processing. The optimized formulation—28.31% protein powder, 20% oil, 1.5% chitosan, 9.99% starch, and 2.03% maltodextrin—achieved a high desirability score and showed excellent agreement between predicted and experimental values, confirming the robustness and predictive utility of the RSM model. The study also demonstrates, for the first time, that lentil proteins form hydrated particulate structures while gluten contributes viscoelastic extensibility, enabling a protein-dense, cohesive matrix uncommon in legume-only cheese analogues. Overall, this work advances the scientific basis for formulating high-protein plant-based cheeses by establishing quantitative, ingredient-specific structure–function relationships. The findings offer actionable strategies for tailoring meltability, texture, and compositional quality and underline the potential of lentil–gluten systems for developing nutritionally balanced and structurally robust dairy-free cheese products. Future research should integrate sensory validation, explore targeted hydrocolloid substitutions to enhance meltability, and assess nutrient bioaccessibility and processing scalability to facilitate commercial application.

CRediT authorship contribution statement

-

Funding

This project has received funding from the European Union's Horizon 2022 research and innovation programme under the Marie Sklodowska-Curie (MSCA-Postdoctoral Fellowships 2022) grant agreement No. 101105558 — NovelPBC — HORIZON-MSCA-2022-PF-01.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Akshit, F., Poswal, V., Kaushik, R., Deshwal, G. K., & Huppertz, T. (2025). Influence of starch on the rheological, textural, and microstructural properties of processed and analogue cheeses. *Encyclopedia, 5*(2), 41. https://doi.org/10.3390/encyclopedia5020041

Alehosseini, A., Wall, C., Segat, A., Tiwari, B. K., Sharma, P., Kelly, A. L., & Sheehan, J. J. (2022). A preliminary evaluation of the impact of pulsed electric field and high-pressure processing treatments on mobility of norbixin molecules through rennet-induced casein matrices. *Journal of Food Process Engineering, 45*(1), e13935. https://doi.org/10.1111/jfpe.13935

Alehosseini, E., McSweeney, P. L. H., & Miao, S. (2025a). Formulation factors influencing the production of dairy-free cheese alternatives. *Journal of Future Foods*. https://doi.org/10.1016/j.jfutfo.2024.10.013

Alehosseini, E., McSweeney, P. L. H., & Miao, S. (2025b). Recent updates on plant protein-based dairy cheese alternatives: outlook and challenges. *Critical Reviews in Food Science and Nutrition*, 1-15. https://doi.org/10.1080/10408398.2025.2452356

Alehosseini, E., Shahiri Tabarestani, H., Kharazmi, M. S., & Jafari, S. M. (2022). Physicochemical, thermal, and morphological properties of chitosan nanoparticles produced by ionic gelation. *Foods, 11*(23), 3841. https://doi.org/10.3390/foods11233841

Ali, F., O'Mahony, J. A., O'Sullivan, M. G., & Kerry, J. P. (2025). Comparative analysis of composition, texture, and sensory attributes of commercial forms of plant-based cheese analogue products available on the irish market. *Foods, 14*(15), 2701. https://doi.org/10.3390/foods14152701

Aydar, E. F., Tutuncu, S., & Ozcelik, B. (2020). Plant-based milk substitutes: Bioactive compounds, conventional and novel processes, bioavailability studies, and health effects. *Journal of Functional Foods, 70*, 103975. https://doi.org/10.1016/j.jff.2020.103975

Bekele, B., Hansen, E. B., Eshetu, M., Ipsen, R., & Hailu, Y. (2019). Effect of starter cultures on properties of soft white cheese made from camel (Camelus dromedarius) milk. *Journal of Dairy Science, 102*(2), 1108-1115. https://doi.org/10.3168/jds.2018-15084

BeMiller, J. N. (2018). *Carbohydrate chemistry for food scientists (3rd ed.).* Cambridge, UK: Woodhead Publishing and AACC International Press.

Bhasney, S. M., Patwa, R., Kumar, A., & Katiyar, V. (2017). Plasticizing effect of coconut oil on morphological, mechanical, thermal, rheological, barrier, and optical properties of poly(lactic acid): A promising candidate for food packaging. *Journal of Applied Polymer Science, 134*(41), 45390. https://doi.org/10.1002/app.45390

Boeck, T., Zannini, E., Sahin, A. W., Bez, J., & Arendt, E. K. (2021). Nutritional and rheological features of lentil protein isolate for yoghurt-like application. *Foods, 10*(8), 1692. https://doi.org/10.3390/foods10081692

Boehm, M. W., Nicholson, R. A., & Baier, S. K. (2023). Designing plant-based analogues of animal-derived foods: a review of research ranging from manufacturing to oral processing. *Current Opinion in Food Science, 50,* 100982. https://doi.org/10.1016/j.cofs.2022.100982

Boye, J. I., Aksay, S., Roufik, S., Ribéreau, S., Mondor, M., Farnworth, E., & Rajamohamed, S. H. (2010). Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. *Food Research International, 43*(2), 537-546. https://doi.org/10.1016/j.foodres.2009.07.021

Boye, J. I., Zare, F., & Pletch, A. (2010). Pulse proteins: processing, characterization, functional properties and applications in food and feed. *Food Research International, 43*(2), 414-431. https://doi.org/10.1016/j.foodres.2009.09.003

Chompoorat, P., Fasasi, A., Lavine, B. K., & Rayas-Duarte, P. (2022). Gluten conformation at different temperatures and additive treatments. *Foods, 11*(3), 430. https://doi.org/10.3390/foods11030430

Chong, S., & Wong, C. (2017). Effect of spray dryer inlet temperature and maltodextrin concentration on colour profile and total phenolic content of Sapodilla (*Manilkara zapota*) powder. *International Food Research Journal, 24*(6), 2543-2548.

Chudy, S., Bilska, A., Kowalski, R., & Teichert, J. (2020). Colour of milk and milk products in CIE *L* a* b** space. *Medycyna Weterynaryjna, 76*(2), 77-81. https://doi.org/10.21521/mw.6327

Cornet, S. H., van der Goot, A. J., & van der Sman, R. G. (2020). Effect of mechanical interaction on the hydration of mixed soy protein and gluten gels. *Current Research in Food Science, 3*, 134-145. https://doi.org/10.1016/j.crfs.2020.03.007

Damodaran, S., Parkin, K. L., & Fennema, O. R. (2007). *Fennema's food chemistry (4th ed.)*: CRC press.

De Angelis, D., Pasqualone, A., Allegretta, I., Porfido, C., Terzano, R., Squeo, G., & Summo, C. (2021). Antinutritional factors, mineral composition and functional properties of dry fractionated flours as influenced by the type of pulse. *Heliyon, 7*(2), e06177. https://doi.org/10.1016/j.heliyon.2021.e06177

Dobson, S., Laredo, T., & Marangoni, A. G. (2022). Particle filled protein-starch composites as the basis for plant-based meat analogues. *Current Research in Food Science, 5*, 892-903. https://doi.org/10.1016/j.crfs.2022.05.006

Dobson, S., & Marangoni, A. (2025). Evaluating the effect of plant protein functionalities on the performance of high-protein plant-based cheese. *Food Chemistry, 492*, 145553. https://doi.org/10.1016/j.foodchem.2025.145553

Dobson, S., & Marangoni, A. G. (2023). Methodology and development of a high-protein plant-based cheese alternative. *Current Research in Food Science, 7*, 100632. https://doi.org/10.1016/j.crfs.2023.100632

Fan, M., Wei, T., Lu, X., Liu, M., Huang, Y., Chen, F., . . . Li, J. (2023). Comprehensive quality evaluation of plant-based cheese analogues. *Journal of the Science of Food and Agriculture, 103*(13), 6595-6604. https://doi.org/10.1002/jsfa.12754

Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. (2017). *Fundamentals of Cheese Science (2nd ed.)*. Boston, MA, USA: Springer.

Garcia-Fontanals, L., Llorente, R., Valderrama, J., Bravo, S., & Talens, C. (2023). Hybrid spreadable cheese analogues with faba bean and mealworm (tenebrio molitor) flours: Optimisation using desirability-based mixture design. *Foods, 12*(7). https://doi.org/10.3390/foods12071522

Gasparre, N., van den Berg, M., Oosterlinck, F., & Sein, A. (2022). High-moisture shear processes: Molecular changes of wheat gluten and potential plant-based proteins for its replacement. *Molecules*, *27*(18), 5855. https://doi.org/10.3390/molecules27185855

Ge, J., Sun, C., Chang, Y., Li, S., Zhang, Y., & Fang, Y. (2023). Understanding the differences in heat-induced gel properties of twelve legume proteins: A comparative study. *Food Research International, 163*, 112134. https://doi.org/10.1016/j.foodres.2022.112134

Grasso, N., Bot, F., Roos, Y. H., Crowley, S. V., Arendt, E. K., & O'Mahony, J. A. (2022). The influence of protein concentration on key quality attributes of chickpea-based alternatives to cheese. *Current Research in Food Science*, *5*, 2004-2012. https://doi.org/10.1016/j.crfs.2022.09.028

Grasso, N., Roos, Y. H., Crowley, S. V., Arendt, E. K., & O'Mahony, J. A. (2021). Composition and physicochemical properties of commercial plant-based block-style products as alternatives to cheese. *Future Foods, 4*. https://doi.org/10.1016/j.fufo.2021.100048

Grasso, N., Roos, Y. H., Crowley, S. V., & O'Mahony, J. A. (2024). Physicochemical properties of plant-based cheese alternatives fortified with calcium. *LWT*, *193*. https://doi.org/10.1016/j.lwt.2023.115657

Hanley, L., Dobson, S., Stobbs, J., & Marangoni, A. G. (2025). Physicochemical and functional characterization of plant protein isolates and their influence on plant-based mozzarella cheese performance. *Food Hydrocolloids, 164,* 111222. https://doi.org/10.1016/j.foodhyd.2025.111222

Hong, G.-P., Wanasundara, J. P., Nickerson, M. T., & Shand, P. J. (2012). *Gelling characteristics of lentil proteins and their contribution to a porcine myofibrillar system.* Paper presented at the 58th International Congress of Meat Science & Technology, Proceedings, Montreal, Canada.

Hoobin, P., Burgar, I., Zhu, S., Ying, D., Sanguansri, L., & Augustin, M. A. (2013). Water sorption properties, molecular mobility and probiotic survival in freeze dried protein–carbohydrate matrices. *Food & function, 4*(9), 1376-1386. https://doi.org/10.1039/C3FO60112A

Hoover, R. (2001). Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers, 45*(3), 253-267. https://doi.org/10.1016/S0144-8617(00)00260-5

Iqbal, A., Khalil, I. A., Ateeq, N., & Sayyar Khan, M. (2006). Nutritional quality of important food legumes. *Food Chemistry, 97*(2), 331-335. https://doi.org/10.1016/j.foodchem.2005.05.011

Jarpa-Parra, M. (2017). Lentil protein: a review of functional properties and food application. An overview of lentil protein functionality. *International Journal of Food Science and Technology, 53*(4), 892-903. https://doi.org/10.1111/ijfs.13685

Jarpa-Parra, M., Bamdad, F., Wang, Y., Tian, Z., Temelli, F., Han, J., & Chen, L. (2014). Optimization of lentil protein extraction and the influence of process pH on protein structure and functionality. *LWT - Food Science and Technology, 57*(2), 461-469. http://dx.doi.org/10.1016/j.lwt.2014.02.035

Jo, Y.-J., Huang, W., & Chen, L. (2020). Fabrication and characterization of lentil protein gels from fibrillar aggregates and the gelling mechanism study. *Food & function, 11*(11), 10114-10125. https://doi.org/10.1039/D0FO02089C

Kamath, R., Basak, S., & Gokhale, J. (2022). Recent trends in the development of healthy and functional cheese analogues-a review. *LWT, 155*. https://doi.org/10.1016/j.lwt.2021.112991

Karaca, A. C., Low, N., & Nickerson, M. (2011). Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International, 44*(9), 2742-2750. https://doi.org/10.1016/j.foodres.2011.06.012

Kaur, M., & Singh, N. (2007). Characterization of protein isolates from different Indian chickpea (*Cicer arietinum* L.) cultivars. *Food Chemistry, 102*(1), 366-374. https://doi.org/10.1016/j.foodchem.2006.05.029

Klupšaitė, D., & Juodeikienė, G. (2015). Legume: Composition, protein extraction and functional properties. A review. *Chemical Technology, 66*(1), 5-12. https://doi.org/10.5755/j01.ct.66.1.12355

Kovačević, J., Bechtold, T., & Pham, T. (2024). Plant-based proteins and their modification and processing for vegan cheese production. *Macromol, 4*(1), 23-41. https://doi.org/10.3390/macromol4010002

Kussmann, M., Abe Cunha, D. H., & Berciano, S. (2023). Bioactive compounds for human and planetary health. *Frontiers in Nutrition, 10,* 1193848. https://doi.org/10.3389/fnut.2023.1193848

Lam, A. C. Y., Can Karaca, A., Tyler, R. T., & Nickerson, M. T. (2018). Pea protein isolates: Structure, extraction, and functionality. *Food Reviews International*, *34*(2), 126-147. http://dx.doi.org/10.1080/87559129.2016.1242135

Lim, T. J., Easa, A. M., Karim, A. A., Bhat, R., & Liong, M. T. (2011). Development of soy-based cream cheese via the addition of microbial transglutaminase, soy protein isolate and maltodextrin. *British Food Journal, 113*(9), 1147-1172. https://doi.org/10.1108/00070701111174587

Liu, H., Xie, F., Yu, L., Chen, L., & Li, L. (2009). Thermal processing of starch-based polymers. *Progress in Polymer Science, 34*(12), 1348-1368. https://doi.org/10.1016/j.progpolymsci.2009.07.001

Lyu, Z., Sala, G., & Scholten, E. (2022). Water distribution in maize starch-pea protein gels as determined by a novel confocal laser scanning microscopy image analysis method and its effect on structural and mechanical properties of composite gels. *Food Hydrocolloids, 133,* 107942. https://doi.org/10.1016/j.foodhyd.2022.107942

Lyu, Z., Sala, G., & Scholten, E. (2023). Melting properties of vegan cheese: Effect of emulsion and protein addition on the thermal behaviour of starch gels. *Food Hydrocolloids, 144,* 108917. https://doi.org/10.1016/j.foodhyd.2023.108917

Martins, S. I. F. S., Jongen, W. M. F., & van Boekel, M. A. J. S. (2000). A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science & Technology, 11*(9), 364-373. https://doi.org/10.1016/S0924-2244(01)00022-X

Masotti, F., Cattaneo, S., Stuknytė, M., & De Noni, I. (2018). Status and developments in analogue cheese formulations and functionalities. *Trends in Food Science & Technology, 74*, 158-169. https://doi.org/10.1016/j.tifs.2018.02.016

Mattice, K. D., & Marangoni, A. G. (2020). Physical properties of plant-based cheese products produced with zein. *Food Hydrocolloids, 105*. https://doi.org/10.1016/j.foodhyd.2020.105746

McClements, D. J. (2015). *Food emulsions: principles, practices, and techniques (3rd ed.)*. Boca Raton: CRC press.

McClements, D. J., & Grossmann, L. (2023). *Next-generation plant-based foods: Design, production, and properties*. Cham, Switzerland: Springer Nature.

Mefleh, M., Pasqualone, A., Caponio, F., De Angelis, D., Natrella, G., Summo, C., & Faccia, M. (2022). Spreadable plant-based cheese analogue with dry-fractioned pea protein and inulin-olive oil emulsion-filled gel. *Journal of the Science of Food and Agriculture, 102*(12), 5478-5487. https://doi.org/10.1002/jsfa.11902

Monga, A., Dev, M. J., & Singhal, R. S. (2022). Cottage cheese from blends of fresh green peas (*Pisum sativum* L.) and dairy milk (*pEaneer*): Preparation, characterization, and sensory evaluation. *LWT, 160*. https://doi.org/10.1016/j.lwt.2022.113263

Naeem, H., Akhtar, A., Akram, N., Suleria, Hafiz Ansar R., & Khalid, N. (2024). Formulation and characterization of lentil milk–based soft cheese analogs: Insights on physicochemical and textural properties. *Legume Science, 6*(3). https://doi.org/10.1002/leg3.248

Ndatsu, Y., Abubakar, H., Aliyu, O. A., Yisa, P. S., Usman, B. H., & Usman, B. H. (2023). Effects of selected natural coagulants on the yield and quality characteristics of glycine max and vigna subterranea milk mixture tofu. *FUDMA Journal of Agriculture and Agricultural Technology, 9*(1), 56-62. https://doi.org/10.33003/jaat.2023.0901.08

Nicolai, T. (2019). Gelation of food protein-protein mixtures. *Advances in Colloid and Interface Science, 270*, 147-164. https://doi.org/10.1016/j.cis.2019.06.006

Pereira, M. T. L., Cavalcante, M. P., da Silva, S. F., Sensheng, Y., Silva, S. d. S., de Aquino, A. C., . . . Frota Gaban, S. V. (2024). Effect of different carrier agents on the yield of powered plant beverage obtained by spray drying and characterization of physicochemical properties. *ACS Food Science & Technology, 4*(10), 2392-2399. https://doi.org/10.1021/acsfoodscitech.4c00478

Rinaldoni, A. N., Palatnik, D. R., Zaritzky, N., & Campderrós, M. E. (2014). Soft cheese-like product development enriched with soy protein concentrates. *LWT - Food Science and Technology, 55*(1), 139-147. http://dx.doi.org/10.1016/j.lwt.2013.09.003

Robertson, J. A., de Monredon, F. D., Dysseler, P., Guillon, F., Amado, R., & Thibault, J.-F. (2000). Hydration properties of dietary fibre and resistant starch: A european collaborative study. *LWT - Food Science and Technology, 33*(2), 72-79. https://doi.org/10.1006/fstl.1999.0595

Rodríguez, A. G. (2017). Vegetable-based cheese and method of making the same (U.S. Patent No. 9,744,345). Google Patents.

Sanders, C., Dobson, S., & Marangoni, A. G. (2024). Effect of saturated and unsaturated fat on the physical properties of plant-based cheese. *Current Research in Food Science, 9*, 100832. https://doi.org/10.1016/j.crfs.2024.100832

Scott, G., & Awika, J. M. (2023). Effect of protein–starch interactions on starch retrogradation and implications for food product quality. *Comprehensive Reviews in Food Science and Food Safety, 22*(3), 2081-2111. https://doi.org/10.1111/1541-4337.13141

Shand, P. J., Ya, H., Pietrasik, Z., & Wanasundara, P. K. J. P. D. (2007). Physicochemical and textural properties of heat-induced pea protein isolate gels. *Food Chemistry, 102*(4), 1119-1130. https://doi.org/10.1016/j.foodchem.2006.06.060

Sharan, S., Zanghelini, G., Zotzel, J., Bonerz, D., Aschoff, J., Saint-Eve, A., & Maillard, M. N. (2021). Fava bean (*Vicia faba* L.) for food applications: From seed to ingredient processing and its effect on functional properties, antinutritional factors, flavor, and color. *Comprehensive Reviews in Food Science and Food Safety, 20*(1), 401-428. https://doi.org/10.1111/1541-4337.12687

Sivakami, U. S. (2021). *Comparing the textural, microstructural and functional properties of dairy and plant-based cheese analogs.* Master's dissertation, Ghent University, Ghent University Academic Repository.

Stevens, C. V. (2020). *Chitin and chitosan: Properties and applications*. Wiley.

Stone, A. K., Karalash, A., Tyler, R. T., Warkentin, T. D., & Nickerson, M. T. (2015). Functional attributes of pea protein isolates prepared using different extraction methods and cultivars. *Food Research International, 76*, 31-38. https://doi.org/10.1016/j.foodres.2014.11.017

Sutter, R., Assad-Bustillos, M., & Windhab, E. (2023). Zein improves desirable melt-stretch properties in plant-based cheeses made from pea protein. *Food Hydrocolloids, 144*. https://doi.org/10.1016/j.foodhyd.2023.108981

Taeger, M., & Thiele, S. (2024). Replacement of milk and dairy products with soy-based alternatives-how to avoid nutrient deficiencies in a milk-free diet? *The Journal of Nutrition, 154*(1), 163-173. https://doi.org/10.1016/j.tjnut.2023.11.003

Talens, C., Llorente, R., Simo-Boyle, L., Odriozola-Serrano, I., Tueros, I., & Ibarguen, M. (2022). Hybrid sausages: Modelling the effect of partial meat replacement with broccoli, upcycled brewer's spent grain and insect flours. *Foods, 11*(21). https://doi.org/10.3390/foods11213396

Tang, Q., Roos, Y. H., & Miao, S. (2023). Plant protein versus dairy proteins: A ph-dependency investigation on their structure and functional properties. *Foods, 12*(2). https://doi.org/10.3390/foods12020368

Tang, Q., Roos, Y. H., & Miao, S. (2024). Comparative studies of structural and thermal gelation behaviours of soy, lentil and whey protein: A pH-dependency evaluation. *Food Hydrocolloids, 146*, 109240. https://doi.org/10.1016/j.foodhyd.2023.109240

Trivedi, D., Bennett, R. J., Hemar, Y., Reid, D. C. W., Lee, S. K., & Illingworth, D. (2008). Effect of different starches on rheological and microstructural properties of (II) commercial processed cheese. *International Journal of Food Science and Technology, 43*(12), 2197-2203. https://doi.org/10.1111/j.1365-2621.2008.01850.x

Ushkalova, A. A., Zhao, R., Gu, Y., Wang, C., & Zhang, T. (2025). Partially substituting cheese base with plant proteins to produce processed cheese: Textural, rheological, microstructural and sensory properties. *Journal of Dairy Science, 108*(5), 4638-4658. https://doi.org/10.3168/jds.2025-26296

Wannasin, D., & McClements, D. J. (2023). Optimizing the appearance of plant-based foods: Impact of pigment and droplet characteristics on optical properties of model oil-in-water emulsions. *Food Biophysics, 18*(2), 289-301. https://doi.org/10.21203/rs.3.rs-2198825/v1

Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiology, 24*(2), 115-119. https://doi.org/10.1016/j.fm.2006.07.004

Wouters, A. G. B., Rombouts, I., Fierens, E., Brijs, K., & Delcour, J. A. (2016). Relevance of the functional properties of enzymatic plant protein hydrolysates in food systems. *Comprehensive Reviews in Food Science and Food Safety, 15*(4), 786-800. https://doi.org/10.1111/1541-4337.12209

Xie, Y., Ding, J., Li, Y., Wei, P., Liu, S., & Yang, R. (2024). The formation of protein–chitosan complexes: Their interaction, applications, and challenges. *Foods, 13*(22), 3572. https://doi.org/10.3390/foods13223572

Ye, A., Hewitt, S., & Taylor, S. (2009). Characteristics of rennet–casein-based model processed cheese containing maize starch: Rheological properties, meltabilities and microstructures. *Food Hydrocolloids, 23*(4), 1220-1227. https://doi.org/10.1016/j.foodhyd.2008.08.016

Younes, I., & Rinaudo, M. (2015). Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Marine Drugs, 13*(3), 1133-1174. https://doi.org/10.3390/md13031133

Zhang, D., Jiang, K., Luo, H., Zhao, X., Yu, P., & Gan, Y. (2024). Replacing animal proteins with plant proteins: Is this a way to improve quality and functional properties of hybrid cheeses and cheese analogs? *Comprehensive reviews in food science and food safety, 23*(1), e13262. https://doi.org/10.1111/1541-4337.13262

Zhang, N.-N., Yang, S., Kuang, Y.-Y., Shan, C.-S., Lu, Q.-Q., & Chen, Z.-G. (2022). Effects of different modified starches and gums on the physicochemical, functional, and microstructural properties of tapioca pearls. *International Journal of Biological Macromolecules, 206*, 222-231. https://doi.org/10.1016/j.ijbiomac.2022.02.143

Figure 1. Schematic representation of the procedures for assessing swelling capacity, water retention capacity (WRC), water solubility index (WSI), and oil-holding capacity (OHC)

Figure 2. Three dimensional (3D) plots of changes in desirability at different levels of (A) protein powder and oil, (B) oil and chitosan, (C) oil and modified tapioca starch, and (D) oil and maltodextrin

Figure 1.

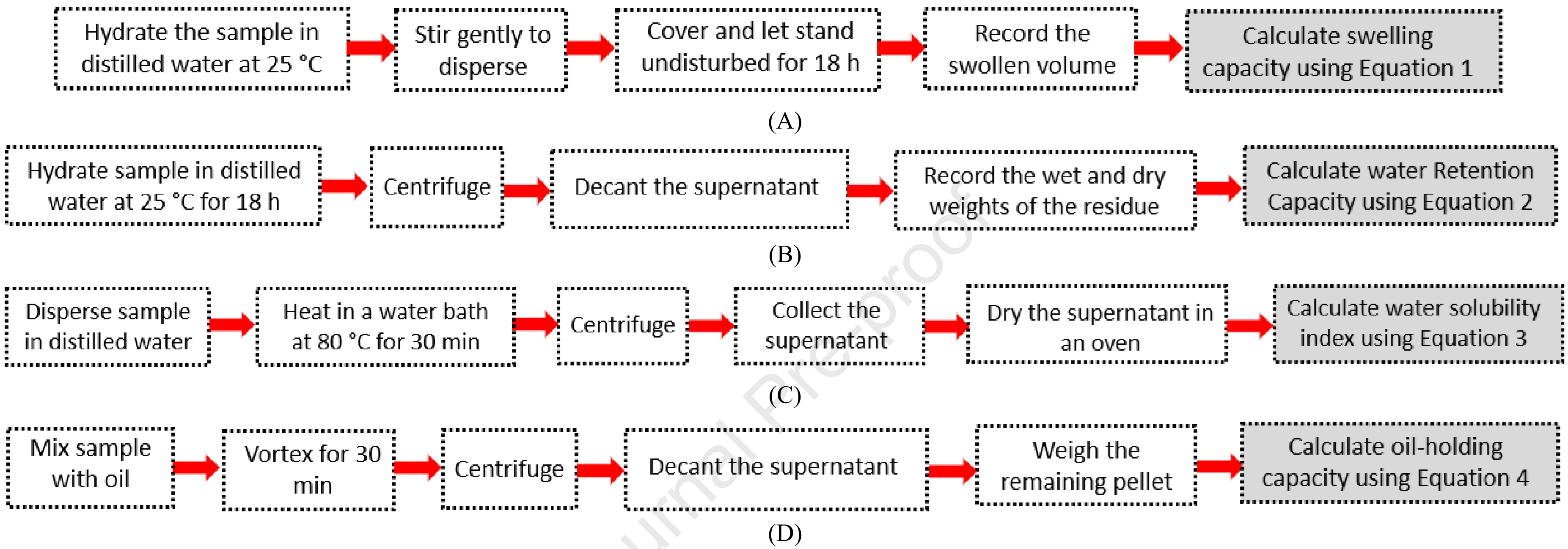
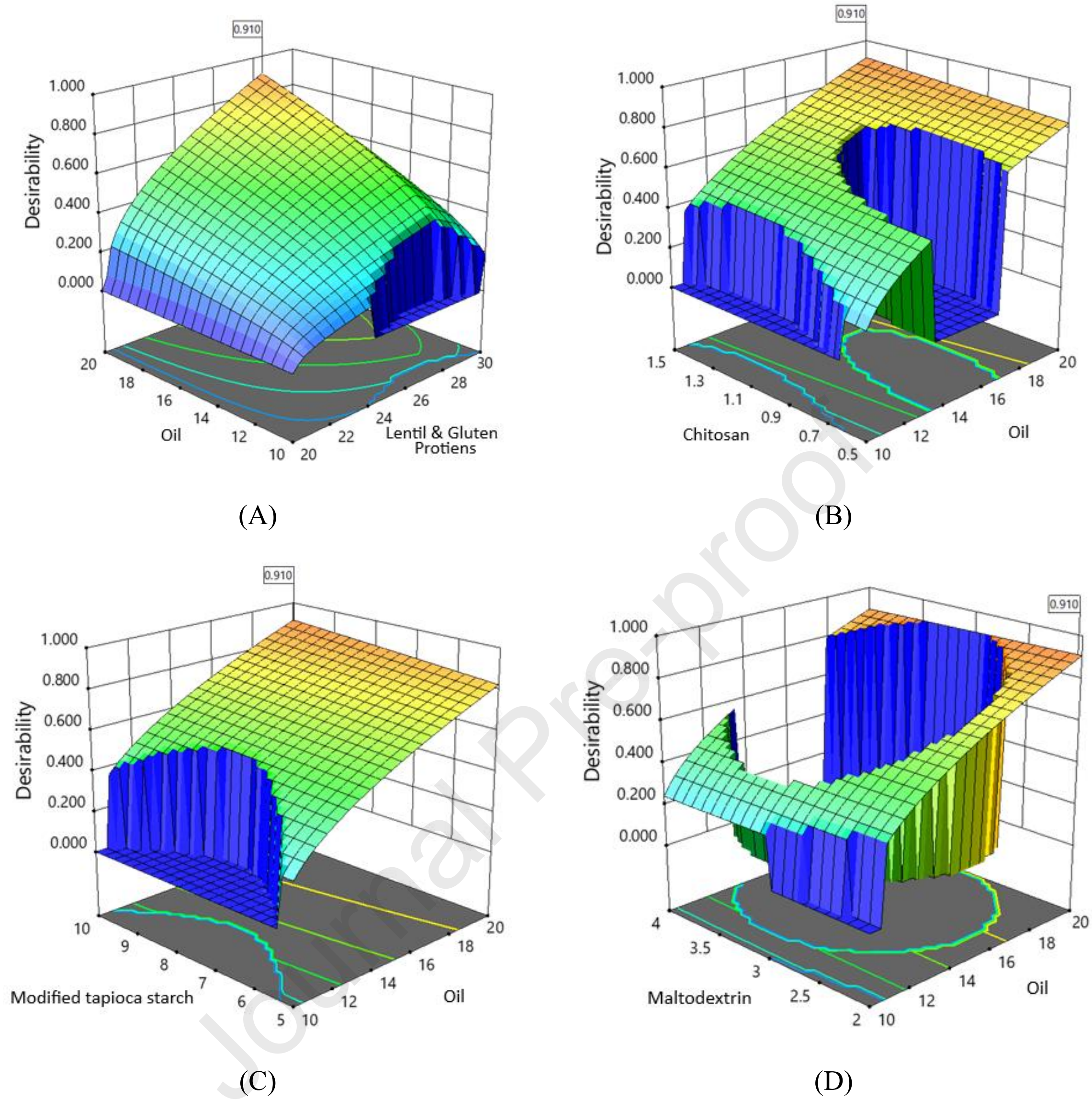

Hydrate the sample in distilled water at 25 °C
Stir gently to disperse
Cover and let stand undisturbed for 18 h
Record the swollen volume
Calculate swelling capacity using Equation 1
(A)
Hydrate sample in distilled water at 25 °C for 18 h
Centrifuge
Decant the supernatant
Record the wet and dry weights of the residue
Calculate water Retention Capacity using Equation 2
(B)
Disperse sample in distilled water
Heat in a water bath at 80 °C for 30 min
Centrifuge
Collect the supernatant
Dry the supernatant in an oven
Calculate water solubility index using Equation 3
(C)
Mix sample with oil
Vortex for 30 min
Centrifuge
Decant the supernatant
Weigh the remaining pellet
Calculate oil-holding capacity using Equation 4
(D)

Figure 2.


0.910
Desirability
1.000
0.800
0.600
0.400
0.200
0.000
Oil
Lentil & Gluten Protiens
Chitosan
Modified tapioca starch
Maltodextrin
(A)
(B)
(C)
(D)

Highlights

- Lentil and gluten proteins form viscoelastic matrices in dairy-free cheese.
- Chitosan and modified tapioca starch enhance hardness and textural stability.
- Oil type modulates meltability by altering protein–starch network dynamics.
- Protein and starch interactions significantly influence color, L^*, a^*, and b^* values.
- RSM modeling predicts physicochemical and textural properties of formulations.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Song Miao reports financial support was provided by European Commission. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.