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Original Article

Investigation of various plant protein ingredients for processed cheese analogues: physical properties and microstructure compared with milk proteins

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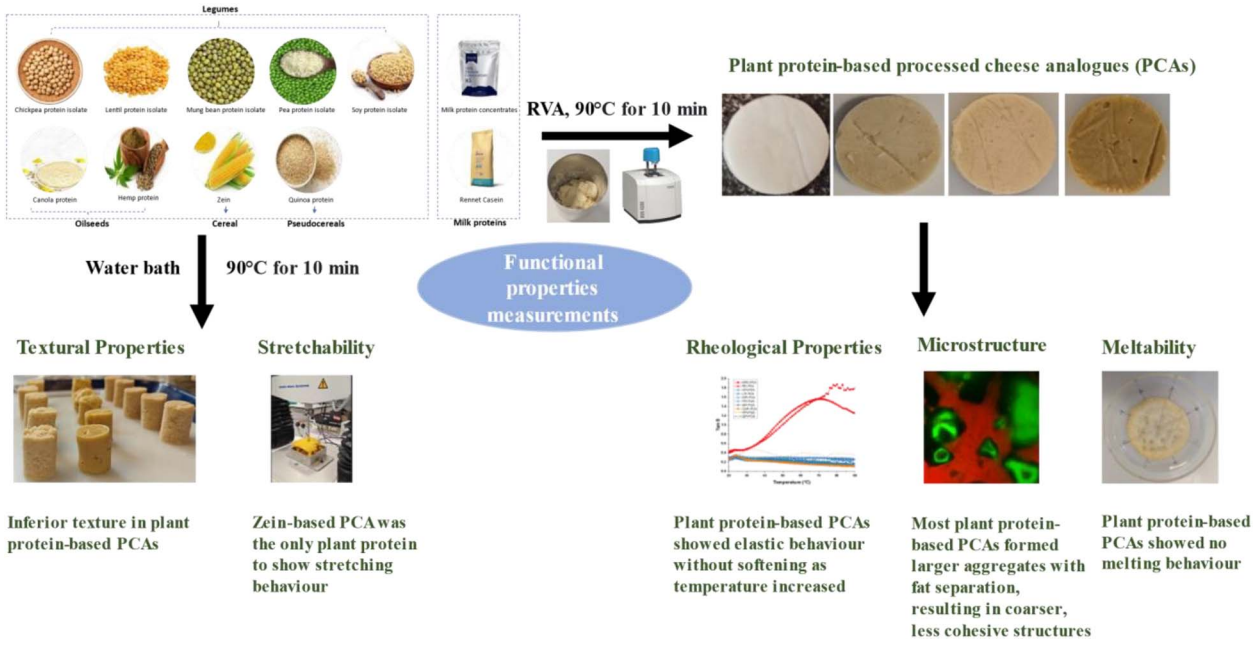
Abstract

This study evaluated various structural and physical properties of several plant proteins in the context of processed cheese analogues (PCAs). A total of 9 plant protein sources were selected to formulate PCA samples. The samples were processed at 90 °C for 10 min using either a rapid visco analyzer or water bath for different tests. Rheological analysis revealed that PCA samples formulated with plant proteins all exhibited solid-like behaviour. PCAs containing legume proteins had a higher storage modulus (G') than that of rennet casein (RC) cheese samples, while canola protein samples showed the lowest G' values. Zein-based PCA had the highest hardness and chewiness but softened when subjected to heat during the stretchability test. In contrast, PCAs containing chickpea, mung bean, or pea proteins exhibited similar hardness to RC-based cheeses but had poorer springiness, cohesiveness, and resilience. Plant protein-based PCAs also lacked melting and stretchability properties due to the absence of a continuous protein network. When ranking all proteins in PCAs based on viscosity, rheological, and textural properties, lentil protein scored the highest, followed by hemp and quinoa proteins, performing most similarly to casein protein. The presented comparison of different plant proteins in PCAs provides valuable insights for cheese analogue development.

Keywords: plant protein-based cheese, texture, microstructure, melt and stretch, rheological properties

Graphical abstract



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Introduction

Cheeses made from animal milk are widely consumed for their exceptional nutritional value. However, the dairy industry faces many challenges, such as the environmental impact of animal farming, animal welfare concerns (Mazac et al., 2023), personal health concerns, and dairy allergies (Espinosa-Marrón et al., 2022; Mazac et al., 2023; Notarnicola et al., 2017; Springmann et al., 2016). Consequently, consumers are increasingly considering plant-based alternatives to replace dairy products, and the global market for plant-based dairy substitutes is projected to continue growing (Fact.MR, 2023).

Plant-based cheese analogues are products prepared from plant ingredients (starch, plant protein, vegetable oil) that are designed to have a similar appearance, texture, and flavour as animal-based cheeses (Grossmann & McClements, 2021). Commercially, many formulations of plant-based cheese rely more heavily on non-protein ingredients, such as starch and coconut oil, to provide structure and functionality to the final product, sacrificing the nutritional value (Grasso et al., 2021, 2022). For instance, the protein content of conventional cheeses (about 18%–25%) is considerably higher than that of plant-based cheeses (0.6%–3%) in the market. Indeed, designing plant-based products with a high protein content that matches the texture, composition, and functionality of conventional cheeses presents significant challenges, mainly due to different protein structures and functional properties (Zhang et al., 2024).

Previous studies have investigated the use of plant proteins as the primary structure to develop plant-based cheese, primarily focusing on soy milk for cream cheese types (Ali et al., 2017; Li et al., 2013, 2020; Zulkurnain et al., 2008) or pea-based cheeses (Ferawati et al., 2021; Masiá et al., 2022; Mefleh et al., 2022; Michel et al., 2022). Zein has been studied and used in plant-based cheeses alone or combined with other plant proteins such as pea or chickpea by several research groups due to its melt-stretch and viscoelastic properties, similar to those of Cheddar cheese (Grasso et al., 2023, 2024; Mattice & Marangoni, 2020a, 2020b; Ozturk et al., 2023; Salgado et al., 2023). Although zein shows promise, its poor solubility, limited nutritional value, flavour issues, and processing challenges make it less desirable for plant-based cheese products (Dobson & Marangoni, 2023). Since a high-protein plant-based cheese with optimal physicochemical properties has yet to be developed, gaining a more comprehensive understanding of the impact of various plant proteins on cheese analogues could be significant for guiding food industry applications. Currently, aside from soy protein cheese analogues, studies on the effects of other plant proteins on the rheological properties, texture, microstructure, and melt and stretch properties of plant-based cheese analogues are limited, and comparative studies investigating the influence of different plant proteins on these properties are scarce (Zhang et al., 2024).

In this study, several plant proteins were selected, including soybeans, peas, chickpeas, mung beans, lentils proteins (legumes), quinoa (pseudocereals), canola, hempseeds (oilseeds), and zein (cereals), to develop processed cheese analogues (PCAs). These were then compared to dairy protein-based cheese analogues containing rennet casein (RC) and milk protein concentrate (MPC) regarding rheological properties, texture, meltability, stretchability, and microstructural characteristics. RC and MPC are commonly used in the PCAs (Warncke et al., 2021). Although RC is the preferred choice due to its intact casein micelle structure, which provides better textural and functional properties, MPC is widely used in PCA manufacturing for its cost-effectiveness (Khiabanian et al., 2022). This preliminary study attempted to uncover the similarities and differences among various cheese analogues made with plant proteins, while also benchmarking them against milk protein-based cheeses. To the best of our knowledge, no previous studies have conducted such a comprehensive comparison. We hypothesize that the plant protein microstructure plays a crucial role in determining cheese analogue properties, providing valuable guidance for selecting ingredients in plant-based cheese products.

Materials and methods

Materials

Rennet casein (RC; SureProteinTM 779) containing 82% protein was obtained from Fonterra Co-operative Group Ltd., New Zealand. Milk protein concentrate (81%; MPC) was obtained from Waiu Dairy Ltd., New Zealand. Soy protein isolate (91%; SPI), pea protein isolate (82%; PPI), and mung bean protein isolate (85%; MPI) were purchased from Bulk Powders New Zealand. Maize zein protein isolate (90%; ZPI) was obtained from Flo Chemical Corp., USA. Lentil protein isolate (88%; LPI) and chickpea protein isolate (83%; CPI) were purchased from Yantai T.full Biotech Co., Ltd., China. Canola protein isolate (CanolaPRO, 90%; CAPI) was purchased from DSM, New Zealand. Hemp protein isolate (70%; HPI) and coconut oil were purchased from Davis Trading Company, New Zealand. Quinoa protein (80%; QPI) was purchased from Qingdao Chibio Biotech Co., Ltd., China. Potassium sorbate was purchased from PureNature NZ, New Zealand. All chemicals used were of analytical grade and were obtained from Sigma Chemical Co., unless otherwise specified.

Preparation of PCA slices

The formulation used for the PCAs was based on Ye et al. (2009) with some modifications. Specifically, the formulation contained 18% protein, 20% coconut oil, 0.5% sodium chloride, 0.5% calcium sulphate, 0.1% potassium sorbate, 2.8% trisodium citrate, 0.7% citric acid, and 57.4% water on a weight ratio basis. To achieve an 18% protein concentration, the protein powder amount was adjusted to accommodate variations in protein content among different sources. The processed cheese slice samples were prepared using a Rapid Visco Analyzer (RVA-4; Newport Scientific Pty Ltd, Warriewood, NSW, Australia), which can mix and heat a 30 g sample in an aluminium with a polycarbonate paddle and was used to make PCAs for measurement of apparent viscosity (Ye et al. 2009). All dry ingredients and oil were manually blended with water for 5 min and hydrated for 1 hr at room temperature. The mixtures were heated to 90 °C over 4 min and held at 90 °C for 10 min, then cooled to 50 °C with mixing at a shear speed of 300 rpm from the heating to the cooling stage. The sample was then rolled into a slice with a uniform thickness of approximately 2.2 mm and sealed in a plastic bag. The slices were immediately cooled to 4 °C in a fridge and stored at 4 °C until further analysis. The preliminary trial showed that zein mixtures underwent liquid phase separation, with the liquid splattering out in the RVA. Therefore, the results for zein from RVA were excluded. The pH of all samples was controlled at 5.4 ± 0.5. The schematic illustration of process and appearances of all samples are shown in Supplementary Figure 1.

Dynamic rheological analysis of PCAs

The rheological properties were measured using a stress-controlled rheometer (Physica MCR 302, Anton Paar, Austria)

equipped with a 20 mm diameter parallel plate with a 2.0 mm gap. Cheese samples were carefully cut into 20 mm diameter discs using a cylindrical cutter and equilibrated at room temperature in a plastic bag for 1 hr before the experiment. The cheese discs were glued to the surface of the lower plate. The upper serrated plate (parallel plate) was lowered until it reached a 2 mm gap distance, and the sample was trimmed and left to rest for 15 min in the rheometer. The exposed edge of the samples was coated with a thin layer of mineral oil to minimize moisture loss during the measurement. The linear viscoelastic range was obtained by performing an amplitude sweep at 1 Hz as the percentage of strain values varied from 0.01% to 100%. The linear region was then selected (0.5% of strain amplitude), and a frequency sweep was performed at 20 °C with frequency varying from 0.01 to 10 Hz. This was followed by a temperature sweep, performed at a constant frequency of 1 Hz and a constant strain amplitude of 0.5%. The temperature was increased from 20 to 90 °C at 3 °C/min using a Peltier heating element. The storage modulus (G'), loss modulus (G"), and loss tangent (tan δ) were determined.

Texture profile analysis

Texture profile analysis (TPA) was performed with TAXT.plus (Stable Micro Systems Ltd., Godalming, Surrey, UK). A 50 mm compression cylinder probe (P/50) attached to a 50 kg load cell was utilized for all TPA measurements. Cheese samples were prepared using the formulation described in section *Preparation of PCA slices* but heated in a water bath at 90 °C for 10 min, producing a larger sample size compared to RVA (which only generated 30 g per sample). The cheese samples were then stored overnight at 4 °C. The following day, the cheese samples were cut into cylinders 20 mm in height and 15 mm in diameter. Two compression–decompression cycles were carried out with a pre-test speed of 1.00 mm/s, test speed of 5.00 mm/s, and post-test speed of 5.00 mm/s, in order to compress the cheese sample to 75% of its initial height. TPA parameters were measured as hardness, fracturability, springiness, chewiness, and resilience. The standard deviation of the results was calculated.

Meltability of PCAs

The meltability of the processed cheeses prepared with RVA was measured using a modified Schreiber melt test (Kosikowski, 1977). Cheese discs of about 5 mm thickness and 30 mm diameter were put on glass Petri dishes and heated in an oven at 170 °C for 10 min. The radii in four directions (0°, 90°, 180°, and 270°) were measured after cooling to 20 °C. The melt index was the average value of the four radius readings. The tests were replicated 4 times.

Stretchability of PCAs

A stretchability test was performed using a TA.HD plus Texture analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK) with a modified cheese stretchability rig including a post comb/fork and a base pot. Only cheese samples containing MPC, RC, zein, and soy protein were tested for stretchability, as the first three proteins have been reported to exhibit stretchability, and the soy protein sample was included for comparison. These samples were prepared using the formulation described in section *Preparation of PCA slices*, but heated in a water bath at 90 °C for 10 min, allowing for the production of a larger sample size. The samples (150 g) were weighed and evenly distributed in the pot. The base pot and post comb with the cheese sample were heated for 15 min in the oven at 95 °C. To minimize drying during heating, the cheese was covered with an aluminium dish. The comb–sample–pot assembly was placed on the TA, and a thermometer (temperature probe) was inserted into the cheese. Once the temperature reached 60 °C, the test was initiated by pulling the comb out of the melted sample. The test speed and distance were set to 10 mm/s and 220 mm, respectively.

Microstructure of PCAs

Confocal microscopy (CLSM) and scanning electron microscopy (SEM) analyses were conducted at the Manawatu Microscopy and Imaging Centre (Massey University, Palmerston North, New Zealand). For CLSM, fresh cheese samples were cut into thin layers and stained 1% Nile Red (1 mg/ml in acetone, to stain fat), and 1% Fast Green FCF (1 mg/ml in Milli-Q water, to stain protein) for 10 min before imaging. Fast Green FCF and Nile Red were excited with He–Ne laser at 633 nm and argon laser at 488 nm, respectively, using a ZEISS LSM 900 microscope (Auty et al., 2001; Gallier et al., 2012). Representative images were captured using a 63× objective lens.

For SEM, all processed cheese samples for SEM were cut into rectangular strips measuring 1 cm × 1 cm × 5 mm—except for quinoa and canola protein samples, as they were soft and could not be fixed and mounted—and were analyzed within 2 weeks of the same day. The cheese strips were fixed by immersing them in a prepared modified Karnovsky's fixative (3% glutaraldehyde and 2% formaldehyde in 0.1 M phosphate buffer at pH 7.2) and washed in phosphate buffer. Next, the samples were dehydrated using a graded series of ethanol washes. Critical point drying was performed using the Polaron E3000 series II critical point drying apparatus (Quorum, Laughton, UK). Samples were mounted onto aluminium stubs using double-sided tape and sputter coated with 100 nm of gold for 110 s using a Baltec SCD 050 sputter coater (Balzers, Liechtenstein). The mounted and coated samples were viewed using an FEI Quanta 200 environmental scanning electron microscope (FEI, Hillsboro, Oregon, USA) at an accelerating voltage of 20 kV and different magnifications (Gunn et al., 2022; Li et al., 2013).

Comparison of plant proteins in PCAs using principal component analysis and TOPSIS

Principal component analysis

Principal component analysis is one of the simplest yet widely used dimensionality reduction algorithms. It linearly decomposes the data into principal components that explain the variances in the data (Hwang & Yoon, 1981). The data preparation and analysis were done in Google Colab using Python 3.10.12, with packages including Pandas, NumPy, and Scikit-learn (sklearn). All plots were created using the Seaborn and Matplotlib libraries. The original data were stored in an Excel file and loaded into the Colab environment using Pandas. Data collected from the rheological analysis and apparent viscosity results were concatenated horizontally, so each row of data represented a single sample. The processed data were then fed into the sklearn PCA model to calculate the normalized top three principal components.

Technique for order of preference by similarity to ideal solution

Technique for order of preference by similarity to ideal solution (TOPSIS) is implemented by establishing an ideal solution and calculating the Euclidean distances of each feature between the samples and the ideal solution. The sample closer to the ideal solution is ranked with a higher score (Fan et al., 2023; Hwang & Yoon, 1981). The rheological, apparent viscosity, and TPA results (without zein) were used for comparison. The missing texture

results for CAPI and QPI were imputed with zeros, indicating no data were acquired. The distances between samples were then calculated using NumPy, with equal weights assigned to all features.

Statistical analysis

All experiments were performed in triplicate (unless otherwise specified). The results are reported as mean values and standard deviations. The data were subjected to one-way ANOVA analysis using SPSS, followed by Tukey's comparison difference. Differences were considered significant at a level of $p < .05$.

Results and discussion

Formation of PCAs

The changes in viscosity during processing in the RVA indicated the mixture of ingredients and formation of the cheese structure, as shown in Figure 1. The data collected during the first 90 s of mixing (when the stirring speed was being increased) are meaningless and, thus, were ignored. However, once the maximum stirring speed of 300 rpm had been reached, useful data were obtained. Specifically, as the temperature increased, the apparent viscosity of the mixtures (paste-like) made with plant proteins consistently remained low and stable. Among the legume proteins, chickpea, lentil, mung bean, pea, and soy showed similar viscosities, while canola, hemp, and quinoa proteins consistently exhibited lower viscosities. In contrast, the RVA curves of the MPC and RC mixtures differed from those of the plant protein mixtures. The apparent viscosity increased with rising temperature due to enhanced hydrophobic interaction between casein molecules, resulting in contraction of the protein network (Lamichhane et al., 2018; Lucey et al., 2003), where RC mixtures contained higher casein and showed higher viscosity than MPC mixtures. MPC mixtures displayed a few peaks during temperature increase, which may be due to the presence of whey protein interrupting casein–casein interactions. RC mixtures exhibited a single peak, with viscosity decreasing sharply thereafter, indicating that the protein network begins to soften after reaching 90 °C. Holding the temperature at 90 °C weakens the casein matrix, although hydrophobic interactions increase, the overall effect may be a weakening of the matrix due to a reduction in the contact area between casein molecules. Additionally, electrostatic repulsion increases while hydrogen bonding decreases (Atik & Huppertz, 2023). The results showed a similar trend as the studies by Prow et al.(Prow & Metzger, 2005) and Kapoor et al.(Kapoor & Metzger, 2005). However, the plant protein mixtures tended to have lower viscosity during mixing and temperature rise, suggesting that they did not undergo significant structural alterations compared to milk protein mixtures (Salunke & Metzger, 2022). Upon cooling to 50 °C, RC and MPC mixtures formed a cheese matrix through the formation of a protein network and had much higher viscosity than plant protein mixtures.

Rheological properties

Frequency sweep test

To better understand the differences in the overall viscoelastic properties of PCAs prepared with different protein sources using the RVA, a frequency sweep test from 0.01 to 10 Hz was conducted within the linear viscoelastic region at a 0.5% strain level. The results are shown in Figure 2. In general, all tested samples exhibited a solid-like behaviour, as indicated by the storage modulus (G') consistently surpassing the loss modulus (G") across the entire frequency range, in agreement with other studies (Rinaldoni et al., 2014; Salgado et al., 2023). This solid-like behaviour implies that the PCAs possess a certain degree of structural integrity. However, there were differences in the magnitudes of G' and G" between the cheese samples derived from plant proteins and those derived from milk proteins (RC and MPC).

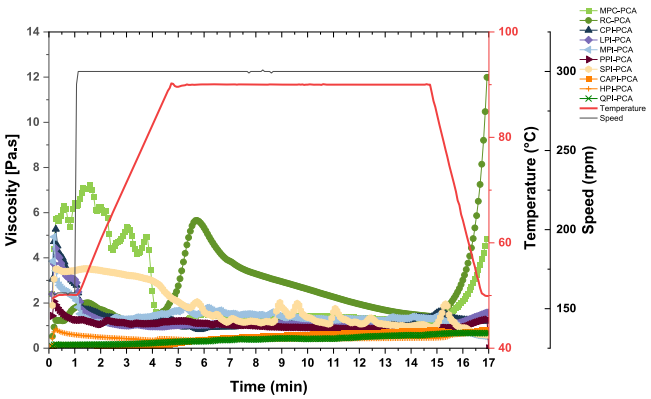

Figure 1. Rapid visco analyzer curves for plant and dairy cheese analogues. PCA = processed cheese analogues; MPC = milk protein concentrate; RC = rennet casein; CPI = chickpea protein isolate; LPI = lentil protein isolate; MPI = mung bean protein isolate; PPI = pea protein isolate; SPI = soy protein isolate; CAPI = canola protein isolate; HPI = hemp protein isolate; QPI = quinoa protein isolate.

From Figure 2A and B, PCAs containing pea, chickpea, mung bean, and soy proteins exhibited relatively high values of G' and G", higher than those of cheese samples formulated with RC. Ben-harb (2018) also reported similar results when they investigated the properties of acid gels made from pure pea, pure milk, and a mixture of pea and milk (1:1) with the aim of creating cheese-like gels with a protein content of 14.8%. Hemp protein, quinoa protein, and lentil protein cheese samples demonstrated similar G' and G" values, which were higher than those of RC samples under lower frequencies (0.01–1 Hz) and remained similar as the frequency increased from 1 to 10 Hz. On the other hand, the cheese samples incorporating canola protein exhibited the lowest values of G' and G", suggesting that canola-protein-based cheeses had weaker gel-like behaviour than the other samples.

The tan δ value measures the ratio of energy lost to energy stored as a function of frequency in processed cheeses containing different protein sources, offering insights into their viscoelastic nature (Figure 2C). A low value of tan δ (<1) indicates a more elastic behaviour, while a high value (>1) indicates a viscous-like behaviour (Mazinani et al., 2020). PCAs with plant proteins exhibited consistent and lower tan δ values across all frequencies, suggesting a predominant elastic nature. Milk protein-based PCA samples (RC and MPC) showed higher tan δ values, indicating both viscosity and elasticity contributions with complex structure and interactions. The mechanical consistency of plant-protein-based PCAs over various frequencies is also evidenced by their shallower G' slopes, as provided in Table 1, ranging from 0.10 to 0.13 (compared to 0.26 and 0.27 for MPC and RC, respectively). These shallower slopes in plant protein-based PCAs indicate a lower frequency dependence, showing that non-covalent bonds including hydrophobic interactions and hydrogen bonds might be controlling the gel network (Zhu et al., 2021), suggesting a more stable and solid-like structure (Beliciu & Moraru, 2013; Grygorczyk et al., 2014; Roesch & Corredig, 2006; Salgado et al., 2023). The consistency in these slope values within plant protein-based PCA samples further implies similar intermolecular interactions and system stability.

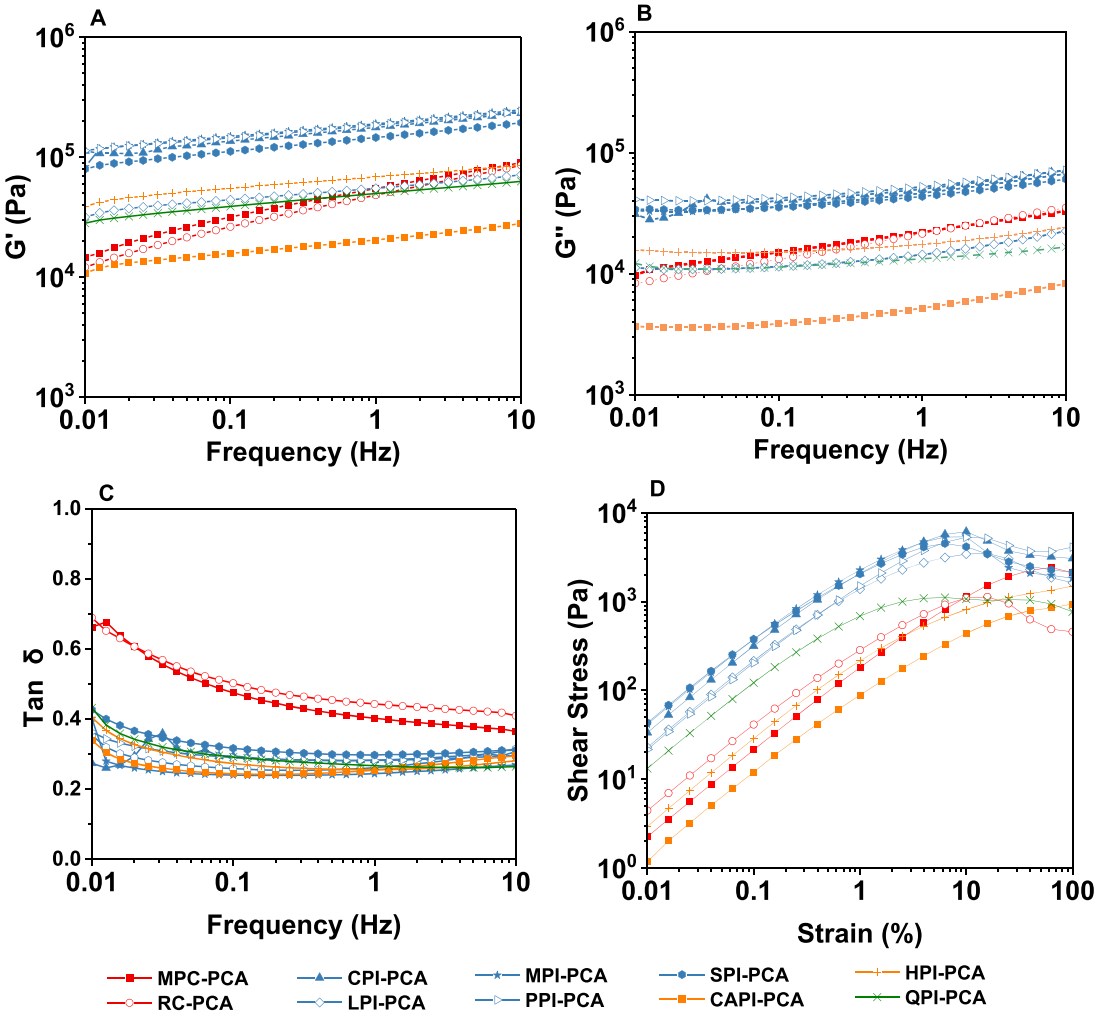

Figure 2. Storage modulus (G') (A), loss modulus (G") (B), and tan δ (C) as a function of the frequency of processed cheese analogues (T = 20 °C, γ = 0.5%). Shear stress–strain curve of processed cheese analogues (D). PCA = processed cheese analogue; MPC = milk protein concentrate; RC = rennet casein; CPI = chickpea protein isolate; LPI = lentil protein isolate; MPI = mung bean protein isolate; PPI = pea protein isolate; SPI = soy protein isolate; CAPI = canola protein isolate; HPI = hemp protein isolate; QPI = quinoa protein isolate. Proteins of the same category are labelled with the same colour.

Table 1. Slopes from log(G')–log(Ω) and log(G")–log(Ω) plots (data obtained from frequency sweep samples at 20 °C and γ = 0.5%).

Slope	MPC-PCA	RC-PCA	CPI-PCA	LPI-PCA	MPI-PCA
G'	0.26 ± 0.01^{a}	0.27 ± 0.01^{a}	0.12 ± 0.01^{b}	0.10 ± 0.01^{b}	0.11 ± 0.01^{b}
G"	0.17 ± 0.02^{a}	0.21 ± 0.01^{a}	0.12 ± 0.04^{e}	0.10 ± 0.02^{bcde}	0.11^{ec}
Slope	PPI-PCA	SPI-PCA	CAPI-PCA	HPI-PCA	QPI-PCA
G'	0.11 ± 0.01^{b}	0.12 ± 0.01^{b}	0.12 ± 0.01^{b}	0.10 ± 0.01^{b}	0.11^{b}
G"	0.09 ± 0.01^{bcde}	0.09 ± 0.01^{bcde}	0.12 ± -0.01^{ec}	0.06 ± 0.01^{d}	0.06 ± 0.01^{d}

Note. Values with the same superscript letter are not significantly different ($p < .05$). G'= storage modulus; G"= loss modulus; Ω= angular frequency; PCA = processed cheese analogue; MPC = milk protein concentrate; RC = rennet casein; CPI = chickpea protein isolate; LPI = lentil protein isolate; MPI = mung bean protein isolate; PPI = pea protein isolate; SPI = soy protein isolate; CAPI = canola protein isolate; HPI = hemp protein isolate; QPI = quinoa protein isolate.

The shear stress values were obtained during amplitude sweeps at T = 20 °C and were plotted against shear strain, as shown in Figure 2D. These stress–strain curves indicate the brittle behaviour of various networks. In this context, the term "brittle" describes the irreversible damage or breakage of the material upon reaching its maximum stress level (Mattice & Marangoni, 2020b). Among plant proteins, brittleness was observed in legume proteins (i.e., chickpea, mung bean, pea, soy, and lentil), with the peak occurring at a strain of 10%. In contrast, other plant proteins, including canola, hemp, quinoa, and milk proteins, demonstrated more ductile material properties.

Temperature sweep test

The rheological melting profiles for PCAs were conducted from 20 to 90 °C at a constant frequency of 1 Hz and a strain of 0.5% on cheese samples after the frequency sweep. The storage modulus (G'), the loss modulus (G"), and Tan δ (G"/G') were measured as a function of temperature, and the results are presented in Figure 3.

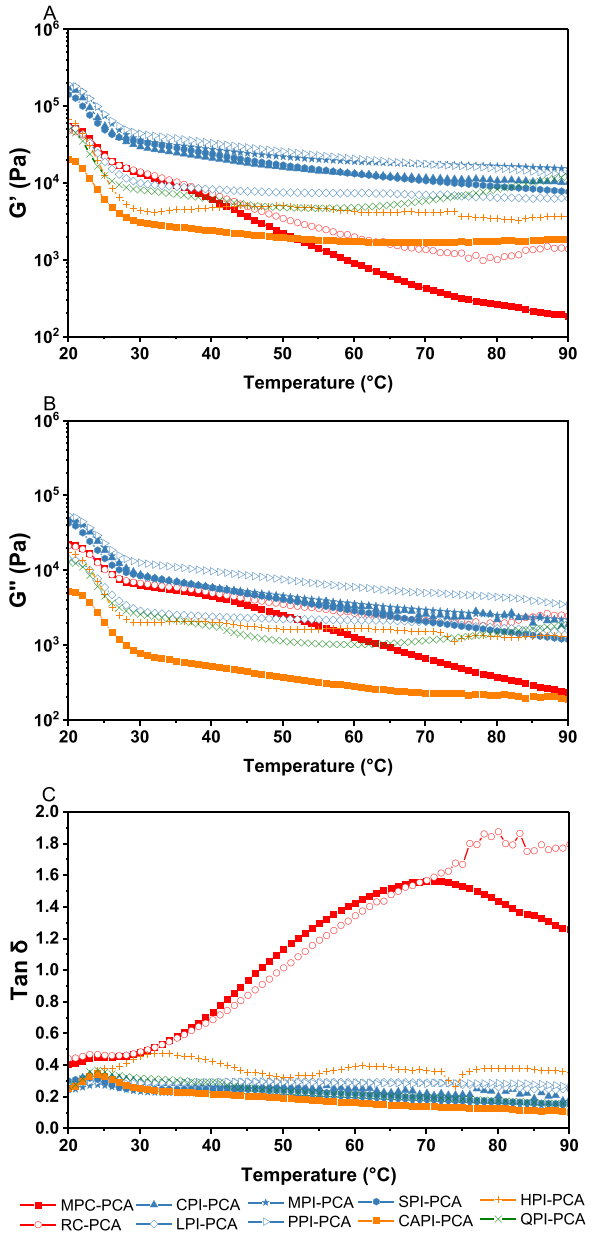

Figure 3. Storage modulus (G') (A), loss modulus (G") (B), and tan δ (C) as a function of temperature of processed cheese analogues ($\Omega = 1$ Hz, $\gamma = 0.5\%$). PCA = processed cheese analogue; MPC = milk protein concentrate; RC = rennet casein; CPI = chickpea protein isolate; LPI = lentil protein isolate; MPI = mung bean protein isolate; PPI = pea protein isolate; SPI = soy protein isolate; CAPI = canola protein isolate; HPI = hemp protein isolate; QPI = quinoa protein isolate. Proteins of the same category are labelled with the same colour.

Milk protein-based (RC or MPC) PCAs and plant protein-based PCAs exhibited vastly different melting profiles. From 20 to 30 °C, all samples showed a significant decrease in G'. This phenomenon can be attributed to the melting of coconut oil, which has a melting point of 24–25 °C (Michel et al., 2022).

For MPC and RC-based PCAs, there was a further decrease in G' (Figure 3A) and G" (Figure 3B) but an increase in tan δ (Figure 3C) as temperature beyond 30 °C, due to a more rapid decrease in the total number and strength of casein–casein bonds in the cheese matrix and a change to a more liquid-like character. Further softening was observed when the PCAs containing MPC and RC reached crossover points (tan $\delta = 1$, G' = G") at around 46–50 °C, indicating that the cheese had transformed from solid to viscous. The difference in melting point can be attributed to protein composition differences. MPC contains a mix of whey proteins and caseins, with whey proteins cross-linking among themselves and with caseins during heating, weakening the casein network. In contrast, the RC cheese analogue forms a stronger protein network. These findings align with Salunke and Metzger (2022), who reported higher melting points for MCC (micellar casein) than MPC in processed cheese. After reaching a peak at 73 and 80 °C for MPC and RC cheeses, respectively, tan δ decreased. This reduction could be attributed to the denaturation of whey protein (70–80 °C), which enhanced protein cross-linking, including the formation of structures between whey protein and caseins (Considine et al., 2007). Additionally there was an increase in hydrophobic-induced casein interaction, which further enhanced elastic behaviour, resulting in the decrease of tan δ values (Atik & Huppertz, 2023; Grasso et al., 2021).

Plant protein-based PCAs did not exhibit softening and viscous behaviour with increasing temperature beyond 30 °C, indicated by the low tan δ value (between 0.2 and 0.4). This observation was in agreement with Dobson & Marangoni (2023) who compared the commercial plant-based cheese alternative with commercial dairy cheese. One possible explanation is that hydrogen bonds played an important role in milk cheese samples, making them more sensitive to temperature changes, while hydrophobic interactions were more dominant in plant protein gels and exhibited greater resistance to temperature changes (Paula Vilela et al., 2020; Zhu et al., 2021). Hemp protein-based PCAs have a slightly higher tan δ than other plant protein-based PCAs, despite showing no melting properties. This could be due to factors such as protein structure and molecular interactions, which would be interesting to investigate further in the future.

Texture profile

The texture parameters of PCAs are presented in Figure 4. The cheese samples containing quinoa protein and canola protein were not measured, as they were too soft and had a sandy texture and, thus, could not be cut into a cylindrical shape like the other samples. Cheese samples containing 18% zein showed the highest hardness (35,616 g) and chewiness (5,055 g), with results that were significantly different from those for other samples (Figure 4A). It has been reported that zein can form a protein network only at temperatures higher than its glass transition temperature (Tg = 28 °C at 20% moisture content) (Salgado et al., 2023). At room temperature, the zein–water network will harden quickly and lose viscoelasticity (Zhang et al., 2022), forming a tough and hard texture. It was suggested that the presence of other proteins (e.g., casein, soy, or pea proteins) or hydrocolloids, or the addition of alkaline agents (calcium hydroxide) could improve the structural functionality of the zein network. This can result in the formation of a viscoelastic network that provides cohesion and imparts a softer texture, as opposed to using zein alone or simply adding it to an ingredient-based mix (Ozturk et al., 2023; Salgado et al., 2023).

Apart from zein-based PCA, cheeses containing milk proteins, chickpea, mung bean, and pea proteins exhibited similar hardness, while lentil and soybean proteins showed lower hardness. The hemp protein PCA had the lowest hardness (290 g). Springiness indicates how well a cheese sample springs back after it has been deformed during the first compression. PCAs containing MPC, RC, mung bean, soy, pea, zein, and hemp proteins had higher springiness than chickpea and lentil samples. Zein-based PCA had the highest chewiness due to its highest

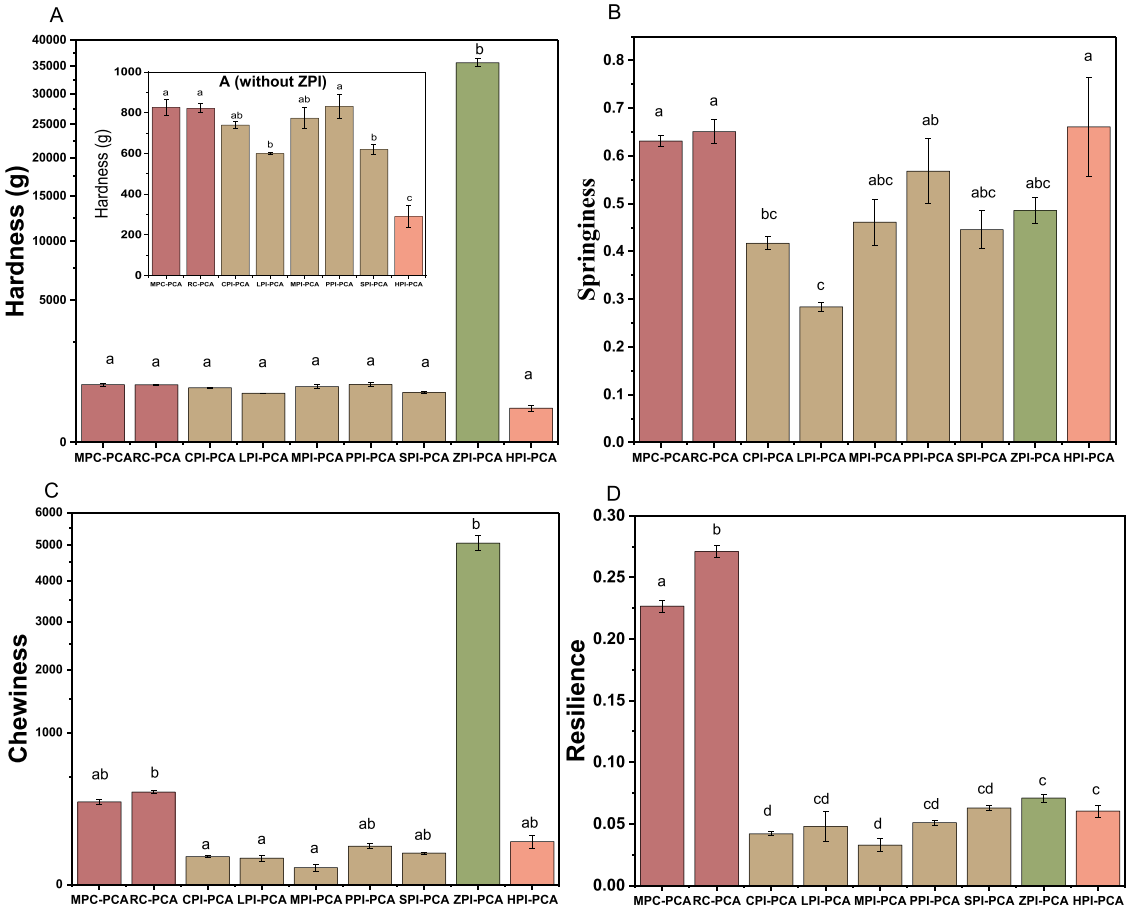

Figure 4. Texture profile of processed cheese analogues containing different proteins: (A) hardness (with and without zein protein isolate), (B) springiness, (C) chewiness, and (D) resilience. No textural properties for canola and quinoa protein-based cheese samples were obtained due to softness. Values with the same letter are not significantly different ($p < .05$). PCA = processed cheese analogue; MPC = milk protein concentrate; RC = rennet casein; CPI = chickpea protein isolate; LPI = lentil protein isolate; MPI = mung bean protein isolate; PPI = pea protein isolate; SPI = soy protein isolate; ZPI = zein protein isolate; HPI = hemp protein isolate. Proteins of the same category are labelled with the same colour.

hardness, which was not desirable for cheese products. MPC and RC cheese samples exhibited moderate chewiness, higher than plant protein-based PCAs. Cheeses containing pea, soy, and hemp proteins also had a moderate chewiness, with no significantly different from milk protein-based PCAs. Resilience shows how well a product resists to regain its original height. It was found that PCA samples containing MPC or RC had higher resilience. Not all samples showed a clear fracture: only PCA samples containing soy protein and pea protein showed fracturability, with the pea protein sample showing higher fracturability (Data not shown).

The moderate hardness (821–826 g) and chewiness (296–370 g), and good springiness and resilience of milk protein-based (RC and MPC) PCAs (lower than that of Zeain-based PCA, but higher than that of others) were possibly due to the formation of a highly uniform and compact protein network (observed in microstructure), which is in agreement with other studies (Mazinani et al., 2021; Salunke & Metzger, 2022). PCAs containing plant proteins showed similar hardness but poor chewiness, springiness, and resilience to milk-based PCAs. This may be due to the formation of large protein aggregates at pH near the isoelectric point or at high ionic strength, making them less resistant to rupture, and less capable of holding water (Andoyo et al., 2018; Rinaldoni et al., 2014). As observed, legume proteins provide higher hardness, closer to casein cheeses, while hemp protein offers greater resilience, chewiness, and springiness. The combination of different plant proteins may yield a better texture profile.

Meltability

Meltability is a critical functional characteristic for processed cheese products, defined as the ability of cheese to flow and spread upon heating, accompanied by a visual loss of integrity of individual cheese shreds (Guinee, 2011). It is primarily influenced by the number and strength of casein–casein interactions (Park et al., 1984). Table 2 presents the melting properties of all the samples. Only the samples containing milk proteins (i.e., MPC or RC) demonstrated meltability, with melt areas of 34.2% and 84.3%, respectively. Furthermore, the RC sample exhibited the greatest extent of diameter expansion, compared to MPC. These results align with those reported by Salunke et al. (2022), who indicated that increasing whey protein content in processed cheese led to textural defects and decreased meltability in cheese products. This is because whey protein can cross-link among themselves as well as with caseins at high temperatures, weakening the casein–casein interactions. The presence of intact casein micelles in RC results in extensive protein–protein and protein–fat globule interactions, forming a fibrous casein network. None of the plant protein samples exhibited any melting behaviour (Supplementary Figure 2), consistent with the observations made by Grasso et al.

Table 2. Mean values ($n = 3$) of the functional properties obtained from the cheese stretchability rig of PCAs containing MPC, RC, SPI, and ZPI.

	MPC-PCA	RC-PCA	SPI-PCA	ZPI-PCA
Max pull force, g	725.24 ± 149.61[a]	548.74 ± 111.45[a]	244.33 ± 18.78[b]	274.54 ± 46.97[b]
Stable point, mm	65.68 ± 11.44[a]	74.52 ± 5.29[a]	35.95 ± 13.44[b]	57.05 ± 3.02[a]
Total work done, g.mm (0-stable point)	11991.31 ± 5127.51[ab]	14875.56 ± 2192.18[a]	5454.19 ± 2943.53[b]	10243.07 ± 1038.12[ab]

Note. Values with the same superscript letter are not significantly different ($p < .05$). PCA = processed cheese analogue; MPC = milk protein concentrate; RC = rennet casein; SPI = soy protein isolate; ZPI = zein protein isolate.

(2021). The authors noted that cheddar cheese exhibited superior meltability, while plant-based products displayed poor meltability due to the lack of a continuous protein network. Although cheese meltability is affected by several factors, including the protein network, moisture-to-protein ratio, moisture-in-non-fat solids, starch content, and the addition of emulsifying salts in the formulation, among these, the protein network has a greater impact than others (Grasso et al., 2021; Zhang et al., 2024).

Stretchability

Stretchability is an important quality attribute of melted cheese. A stretchability test was conducted on the milk protein-based PCA samples with melting properties (tan $\delta > 1$) and zein-based samples (based on Mattice & Marangoni (2020b)). Furthermore, soy protein (without melting properties) was added for comparison. PCAs were prepared using the method described in section *Stretchability of PCAs*. The stretchability results, including the maximum pull force, total work done (the area from the initial pull to the stable point), and stable stretch distance, were used as parameters to compare the stretching of the samples (Table 2 and Figure 5). Due to the limited sensitivity of the texture analyzer, it was unable to detect the stretch breaking point of the samples. In this context, the relatively stable point (gradient ~0) was employed as an indicative measure of the stretch distance. In cheese, stretchability refers to the ability of the casein network to maintain its integrity without breaking when it is pulled. This depends on intact casein to maintain the strength of the strands with enough calcium and phosphorus (Lucey et al., 2003; Lucey & Fox, 1993). It is also affected by how the casein molecules stick together and handle energy (Lucey et al., 2003). The stretch test requires casein molecules to be close, yet flexible enough to quickly change shape (Lucey & Fox, 1993).

In comparison, PCA containing milk proteins (i.e., RC or MPC) exhibited higher stretchability than plant proteins, characterized by higher maximum pull force, total work done, and stretch distance. MPC showed slightly lower stretch distance and total work done than RC, possibly due to the presence of whey proteins interacting with casein, which may disrupt the casein–casein network. This result aligns with Salunke et al'.s study (2022), where cheese samples containing micellar casein (MCC) exhibited greater stretch than the MPC sample. The study explained that the strands in MPC cheese samples proved to be fragile, breaking shortly upon fork lifting, whereas the MCC cheese samples displayed longer strands, indicating that the presence of whey proteins negatively influenced the cheese's stretchability.

Previous studies have indicated that cheese made with zein demonstrated stretchiness (Grasso et al., 2023; Mattice & Marangoni, 2020b). This is because the zein networks become softer at higher temperatures due to weakened non-covalent interactions, resulting in improved stretchiness. PCAs containing zein exhibited some level of stretchability, although it was lower than that of cheese samples formulated with RC, as evidenced by lower maximum pull force ($p < .05$), total work done, and stretch distance. The PCA formulated with soy protein exhibited the lowest maximum pull force, total work done, and shortest stretching distance, which was significantly lower than that of the RC sample. The absence of melting behaviour in soy protein (representing all other plant proteins) limited its ability to stretch. The samples could not form a molten mass, which is necessary for the subsequent stretching process, as evidenced by the accompanying photographs (Supplementary Figure 3). It is reasonable to speculate that other PCAs containing various plant proteins (e.g., pea, chickpea, and mung bean) lacking melting properties would similarly lack stretchability. The comparative analysis of PCAs containing zein, RC, MPC, and soy protein highlights the role of melting properties in determining their stretchability.

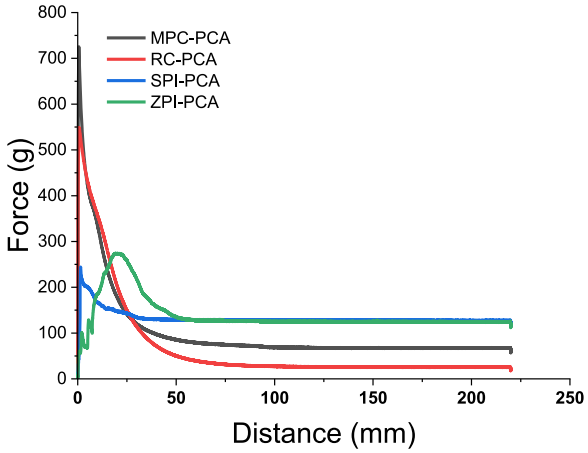

Figure 5. Stretchability derived from texture profile analysis of processed cheese analogues (PCA) containing rennet casein (RC), milk protein concentrate (MPC), soy protein isolate (SPI), and zein protein isolate (ZPI).

Microstructure

The CLSM visualized the distribution of coconut oil and different proteins in PCAs (Figure 6A). A homogeneous casein network and uniform distribution of spherical fat droplets (<20 μm) throughout the casein network were observed in the milk protein-based PCAs, consistent with the findings of Ye et al. (2009) and Noronha et al. (2008) for imitation cheeses made with RC and commercially processed cheeses by Ramel (2017). However, in most plant protein-based PCAs, including lentils, mung bean, pea, soy, hemp, and canola proteins, the fat droplets formed non-spherical pools or coalesced, leading to fat and protein phase separation. This suggests that plant proteins generally have poorer emulsifying properties than milk proteins. In contrast, chickpea-based and quinoa-based PCAs exhibited smaller, spherical fat droplets similar to those in MPC and RC, indicating that different plant proteins

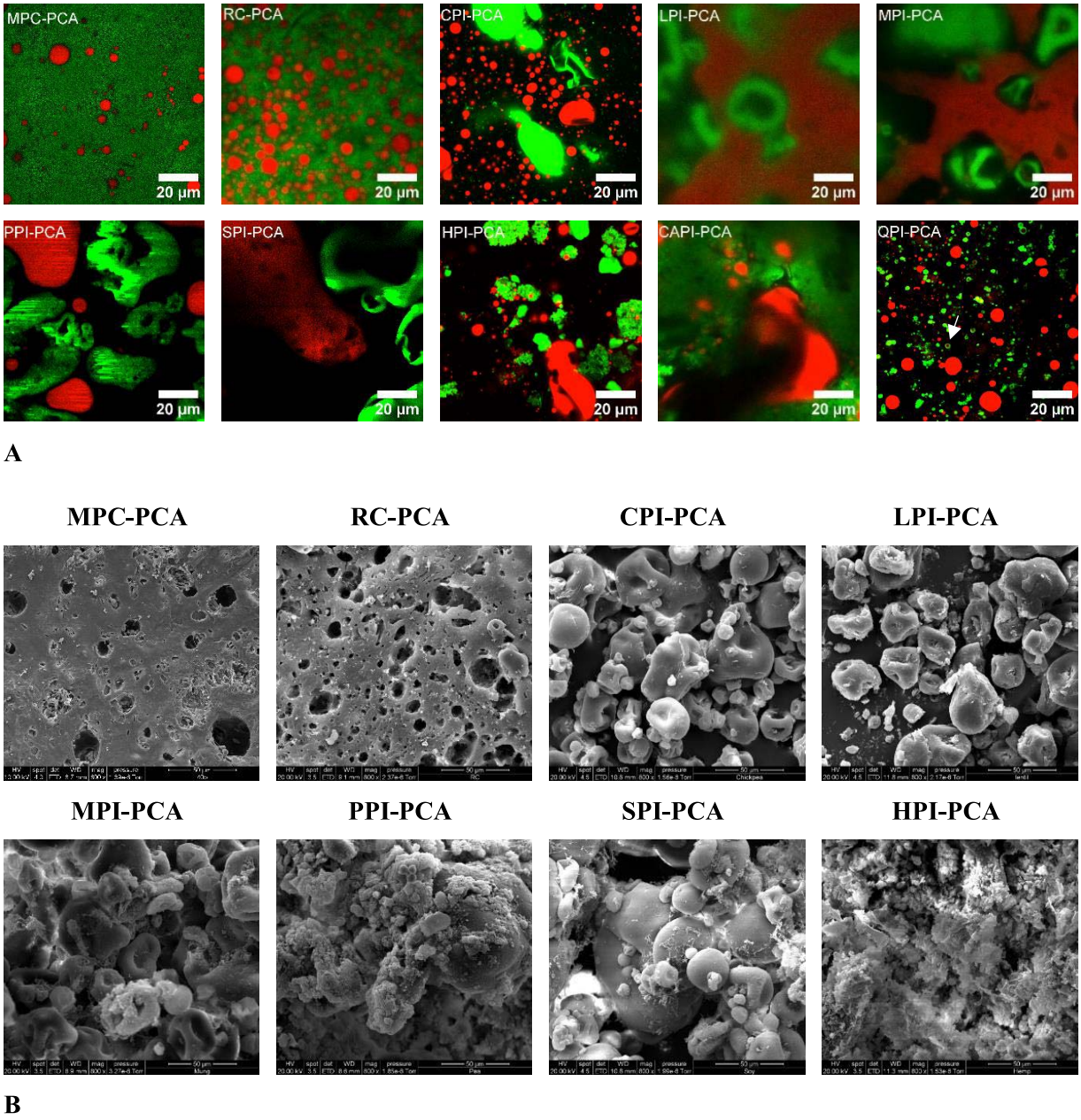

Figure 6. Confocal micrographs (CLSM) (A, top) with a scale bar of 20 μm and scanning electron microscope (SEM) (B, bottom) with a scale bar of 50 μm and magnification of 800 of processed cheese analogues containing different protein sources. Fat was stained in red, and protein was stained in green for CLSM analysis. No SEM for canola and quinoa protein-based samples. PCA = processed cheese analogue; MPC = milk protein concentrate; RC = rennet casein; CPI = chickpea protein isolate; LPI = lentil protein isolate; MPI = mung bean protein isolate; PPI = pea protein isolate; SPI = soy protein isolate; HPI = hemp protein isolate, CAPI = canola protein isolate; QPI = quinoa protein isolate.

have varying emulsifying properties. Interestingly, the microstructure of the cheese sample containing quinoa protein revealed that some fat globules were stabilized by quinoa protein (white arrow in Figure 6A) (Cui et al., 2023; Lingiardi et al., 2022). Moreover, plant protein-based cheeses tended to form larger protein aggregates with a non-continuous network, with legume proteins forming larger aggregates than hemp, canola, and quinoa proteins. Among these, canola protein aggregates appeared even more diminutive and denser, similar to those in the milk-protein-based PCAs.

The SEM analysis revealed uniform and smooth microstructures with an open and fibrous protein matrix in cheese samples made with MPC and RC (Figure 6B). Mounsey and O'Riordan (2001) have reported the similar observation for processed cheese. However, the whey protein present in MPC samples adhered to the surface of the casein, resulting in rough and irregular protein aggregates, leading to a coarser structure (Atia et al., 2004). However, larger protein aggregates and a more irregular microstructure were observed in the plant protein-based PCAs, with legumes forming large clusters (>50 μm), while hemp protein formed fragmented structures with large, loosely packed aggregates. Additionally, larger pores and gaps were observed in PCAs containing chickpea, lentil, and mung bean proteins, which could be attributed to their being less resilient to deformation.

In summary, both CLSM and SEM evidenced that the milk protein-based PCAs showed the most uniform distribution of fat droplets within the protein matrix, resulting in better textural

Table 3. The scores of processed cheese analogues containing different proteins were evaluated using apparent viscosity, dynamic rheological measurements, and textural profile analysis results (without zein).

PCAs	RC	MPC	LPI	HPI	QPI	SPI	CPI	PPI	MPI	CAPI
Score	1	0.812	0.662	0.645	0.612	0.565	0.552	0.547	0.546	0.040

Note. PCAs = processed cheese analogues; RC = rennet casein; MPC = milk protein concentrate; CPI = chickpea protein isolate; LPI = lentil protein isolate; MPI = mung bean protein isolate; PPI = pea protein isolate; SPI = soy protein isolate; HPI = hemp protein isolate, CAPI = canola protein isolate; QPI = quinoa protein isolate.

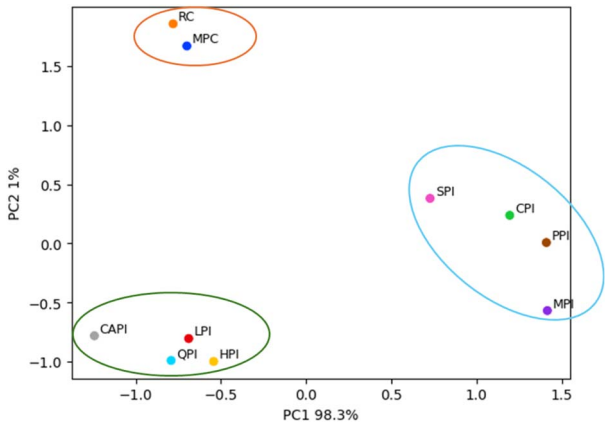

Figure 7. Principal component analysis of processed cheese analogues containing different proteins was conducted using apparent viscosity and dynamic rheological results. MPC = milk protein concentrate; RC = rennet casein; CPI = chickpea protein isolate; LPI = lentil protein isolate; MPI = mung bean protein isolate; PPI = pea protein isolate; SPI = soy protein isolate; HPI = hemp protein isolate, CAPI = canola protein isolate; QPI = quinoa protein isolate.

and physical properties. In contrast, most plant protein-based PCAs exhibited larger protein aggregates with a non-continuous network, and irregular fat droplets were separated from the protein network, resulting in poor emulsifying properties. They did not form as cohesive a network as milk proteins, resulting in a rougher, coarser, and more uneven structure, similar to observations in other studies (Grasso et al., 2021; Li et al., 2013). Larger plant protein aggregates may contribute to relatively higher viscosity during mixing and increased rigidity and stiffness in PCAs, as observed in legume protein-based PCAs. Conversely, smaller aggregates may result in lower viscosity during mixing and softer, sandier textures in PCAs, as seen in canola and quinoa protein-based PCAs.

Comparison of plant proteins in PCAs using principal component analysis and TOPSIS

The datasets from the apparent viscosity and dynamic rheological measurements were subjected to principal component analysis for data visualization and dimension reduction. As shown in Figure 7, the scatter plots represent the first two components, with 98.3% of the variance explained by the first component (PC1) and 1% by the second component (PC2). A distinct separation and clustering among proteins were observed. MPC and RC are closely grouped, while CAPI, QPI, LPI, and HPI form another group, and SPI, CPI, PPI, and MPI form a third group. This suggests that proteins within the same group have similar rheological and viscosity properties, but they are significantly different from those in other groups.

Table 3 ranks the PCAs containing different proteins using TOPSIS, based on the apparent viscosity, dynamic rheological measurements, and textural profile analysis results (excluding zein). RC was set as the ideal solution, receiving a score of 1. As expected, MPC closely followed, indicating its similarity to the ideal solution. PCAs formulated with LPI, HPI, and QPI exhibited moderate performance, with scores ranging from 0.662 to 0.612. Conversely, PCAs containing SPI, CPI, PPI, and MPI scored between 0.565 and 0.546, indicating less optimal performance. However, CAPI cheese demonstrated the lowest score, indicating the least favourable similarity to the ideal solution. These findings are consistent with the principal component analysis results and provide valuable insights into identifying plant proteins that are most suitable for producing PCAs with desirable textural and rheological properties to RC.

Conclusion

This study brought together new information on the differences in the microstructure and physical properties of PCAs formulated with different plant proteins. Plant protein-based PCAs lacked melting and stretching behaviour, mainly due to the lack of a continuous protein network like casein. Legume protein-based cheeses exhibited similar hardness to RC-based cheeses but displayed inferior springiness, cohesiveness, and resilience, likely due to the formation of larger protein aggregates and poor oil-emulsifying ability. In contrast, hemp protein formed aggregates of varying sizes, canola and quinoa proteins tended to create smaller and more uniform aggregates, leading to a softer texture with lower elasticity in cheeses.. Based on TOPSIS, lentil protein ranked the highest scores in PCAs compared to other plant proteins, showing performance most similar to milk proteins. PCA containing zein, on the other hand, exhibited distinct characteristics; in particular, they displayed softening properties when heated and had the hardest texture at room temperature. This information provides insight into the differences in physical properties and microstructure between various plant protein ingredients in PCAs. It guides the selection of plant proteins for developing high-protein plant-based cheeses.

Supplementary material

Supplementary material is available at *International Journal of Food Science and Technology* online.

Author contributions

Di Lu (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Investigation [equal], Methodology [equal], Writing—original draft [equal]), Debashree Roy (Supervision [equal], Writing—review & editing [equal]), Alejandra Acevedo-Fani (Supervision [equal], Writing—review & editing [equal]), Harjinder Singh (Supervision [equal], Writing—review & editing [equal]), and Ye Aiqian (Conceptualization [equal], Funding acquisition [equal], Project administration [equal], Supervision

[equal], Writing—review & editing [equal]). The data that support the findings of this study are available from the corresponding author, Aiqian Ye, upon reasonable request.

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Conflicts of interest

The authors declare no conflicts of interest.

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