Food Chemistry 434 (2024) 137434

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Hybrid Paneer: Influence of mung bean protein isolate (*Vigna radiata* L.) on the texture, microstructure, and *in vitro* gastro-small intestinal digestion

Shince Tojan [a], Lovedeep Kaur [b], Jaspreet Singh [b,*]

[a] *School of Food and Advanced Technology, Massey University, Palmerston North, New Zealand*
[b] *Riddet Institute, Massey University, Palmerston North, New Zealand*

ARTICLE INFO

Keywords:
Cow milk
Mung bean protein
Vigna radiata
Paneer, cheese analogues
Rheology
Texture
In vitro protein digestibility

ABSTRACT

Replacing dairy proteins with legume proteins such as mung bean protein can create hybrid cheese alternatives with superior nutritional and functional properties. The effects of partially replacing (30%) cow milk with mung bean protein isolate (MBPI) on the rheology, texture, microstructure, and digestibility of paneer (acid-heat coagulated cheese) were studied. The developed hybrid cow milk-mung bean paneer (CMMBP) had higher protein and moisture contents, lower fat content, and a darker colour than cow milk paneer (CMP). CMMBP showed a significant reduction in hardness, cohesiveness, chewiness, and springiness compared to the cow milk-based control. Frequency sweeps performed using a dynamic rheometer showed higher storage modulus (G′) for CMMBP compared to CMP, indicating greater elastic properties of the hybrid paneer. In vitro digestibility of CMMBP was significantly lower than CMP, as shown by the lower overall ninhydrin-reactive free amino N release and the presence of resistant peptides at the end of digestion.

1. Introduction

Paneer is a type of fresh, soft, unripened cheese made by the acid-heat coagulation of milk. It is quite popular in the Indian sub-continent and is used to prepare various culinary dishes and snacks. Paneer is highly nutritious and is rich in proteins, fats, vitamins, and minerals like calcium and phosphorous (Kumar et al., 2014). The global paneer market size reached US$ 9.4 Billion in 2022 and shows an increasing future market trend. The growing population, changing dietary habits, exploration of intercultural foods, and introduction of paneer in Western fast foods are significant factors behind the increasing global demand for paneer (IMARC, 2023).

Despite the growing demand, there is a gradual decline in the use of dairy-based products due to changing customer lifestyles and health concerns associated with the consumption of milk products, such as various allergic reactions in specific individuals, antibiotic residues in milk, and cholesterol. However, due to the rise in population, there is a massive demand for proteins, leaving industries looking for more sustainable and innovative alternative protein ingredients (Boland et al., 2013).

Using more affordable, accessible, and nutritious substitutes like legume proteins can effectively overcome the global protein challenge. Mung bean (*Vigna radiata* L.) belongs to the family of legumes and has a protein content of 20–27 %. Mung bean protein has a similar amino acid profile to soybean protein and matches well with FAO/WHO reference protein (Brishti et al., 2017). The allergenicity of mung bean is comparatively lower and was found to decrease in the order of peanut > soybean > lentil > chickpea > pea > mung bean (Verma et al., 2013). Researchers have also reported the highest yield of bean curd produced using mung bean protein compared to other legume proteins (Cai et al., 2002). Mung bean protein's gelling capacity was found to be better than soy protein due to its lower least gelation concentration (LGC) (Brishti et al., 2017). Motoyama and Ashida (2014) reported that the addition of 8.3 %–15 % mung bean protein isolate can provide a good gel that can be further enhanced by the addition of sodium chloride. The above-mentioned characteristics suggest that Recently, Ouyang et al. (2022) investigated the potential of plant proteins to be used as an ingredient to develop cheese with characteristic sensory properties tailored toward Southeast and East Asian customers. They also suggested using proteins such as mung beans that are common to the Southeast and East Asian population as it may be easier for them to accept familiar flavours. A recent review by Hou et al. (2023) also suggests that mung bean proteins are promising bioactive ingredients and also have a vast potential to be used in different value-added food products, such as meat analogues,

---

* Corresponding author.
E-mail address: j.x.singh@massey.ac.nz (J. Singh).

https://doi.org/10.1016/j.foodchem.2023.137434
Received 7 April 2023; Received in revised form 20 August 2023; Accepted 7 September 2023

plant-based yoghurts, emulsion gels and egg substitutes.

Partial replacement of dairy products with legume proteins is of high importance as it provides an opportunity to develop hybrid dairy-legume cheese analogues that can open the door for new markets and the development of innovative food products with low environmental impact along with improved nutritional and functional properties. However, no studies have reported the use of mung bean protein in hybrid food products like cheese, paneer etc. Therefore, the present study was aimed at developing a hybrid dairy-mung bean protein-based paneer (CMMBP). The textural, rheological, microstructural properties of the hybrid paneer were evaluated and compared with a cow milk-based paneer (CMP). Knowledge of the difference between the kinetics of protein digestion in a single system and a hybrid system is essential for manufacturing products with high nutritional and biological value (Kaur et al., 2022). Hence, the cow milk-based paneer and the hybrid paneer were also compared for their gastro-small intestinal protein digestion characteristics using an *in vitro* model.

2. Materials and methods

2.1. Materials

Standard milk, with protein, fat, and carbohydrate contents of 3.3, 3.4, and 4.8 %, respectively, and citric acid were purchased from a local supermarket (PAK'n Save, Palmerston North, New Zealand). Mung bean protein isolate (Harbin Hada Starch Co. Ltd., Wuchang, China), with protein, fat, and carbohydrate contents of 71 %, 0.6 %, and 19.3 %, used for the study was purchased from Davis trading company, Palmerston North.

The enzymes required for the *in vitro* digestion were obtained from Sigma-Aldrich Pty Ltd (USA). Milli-Q water was used for the preparation of reagents and the experiments. All the chemicals and reagents used for this study were of analytical grade.

2.2. Methods

2.2.1. Preliminary studies

The first phase of this study involved identifying the potential plant protein isolate from soy, pea, hemp, mung bean, and plant milk like oat, rice, cashew, and soymilk for partially replacing cow milk for the manufacturing of hybrid paneer. Different ratios of cow milk to plant protein (100:0, 90:10, 80:20, 70:30, 50.50, 30:70, and 100:0) were investigated to identify the plant protein's potential to replace cow milk for the manufacturing of paneer partially and 70: 30 ratio was selected for further experimentation. Mung bean protein was chosen considering cost, availability, and its ability to form curd when acidified with citric acid. The selection criteria and results for the preliminary experimentation are discussed in section 3.

2.2.1.1. Preparation of cow milk paneer (CMP). Paneer was prepared by slightly modifying the method of Laursen et al. (2022). The milk was heated to a temperature of 90 °C and was allowed to cool down to 80 °C. Citric acid solution (1 %) was heated to 70 °C and added to the milk with continuous stirring until clear whey separated. The formed curd was rested for 5 min, ensuring the temperature did not fall below 63 °C. The curd was then drained and separated using a muslin cloth and was pressed using a cheese press by applying a weight of 5 kg for 20 min. The pressed curds were then immersed in chilled water at 4 °C for 2 h. The paneer was removed from the chilled water and was allowed to drain off the excess water. The paneer was then wrapped using film wrap and was stored at refrigerated conditions (4 °C).

2.2.1.2. Preparation of cow milk-mung bean paneer (CMMBP). Mung bean protein suspension was prepared by adding MBPI to water (3.3 % protein) with continuous mixing using a magnetic stirrer for 30 min at 400 rpm at room temperature. The cow milk-mung bean paneer was made by adding mung bean protein suspension to cow milk. The rest of the steps were similar to the preparation of cow milk paneer (**Supplementary data Figure S1**).

2.2.1.3. Preparation of mung bean curd for confocal microscopy. The mung bean curd was prepared in the same way as cow milk paneer (section 2.2.2): by heating mung bean suspension (3.3 % protein) to 90̊C, then cooling it to 70 °C and coagulating using 1 % citric acid solution. This mung bean curd was used in the microstructural image analysis using confocal microscopy (Leica SP5 DM6000B, Leica Microsystems, Heidelberg, Germany).

2.2.1.4. Proximate composition and colour analysis. The moisture content of the paneer samples was determined using the hot air oven method (AOAC, 2016). The total protein content of the paneer samples was determined using the Kjeldahl method (AOAC, 2016) using a nitrogen-to-protein conversion factor of 6.38. The fat content of the paneer samples was estimated using the Soxhlet extraction method (AOAC, 2016). All the analyses were conducted in triplicate.

The colour profile of the paneer samples was analysed using a Minolta Chroma meter CR 400. The L* (indicates lightness range between 0 and 100), a* (Positive a* value = redness and negative a* value = greenness), and b* (positive b* value = yellowness and negative b* value = blueness) parameters were determined according to CIELAB colour space. The equipment was calibrated using a white tile (Y = 86.6, x = 0.3162, y = 0.3232) as a standard. Five measurements were taken per sample.

2.2.1.5. Small amplitude rheology. Small amplitude oscillatory measurement was performed on paneer samples using a controlled stress rheometer (Anton Paar, MCR302, Germany), according to the method given by Hosseini-Parvar et al. (2015). Two paneer samples were analysed for various rheological properties using a rheometer attached with a 25 mm diameter parallel plate geometry and a gap size of 2 mm. A cylindrical piece of the sample (2 mm thickness) was cut carefully using a mould and wire cutter and was stored in an airtight plastic bag to prevent dehydration. A thin layer of mineral oil was applied around the sample to avoid evaporation. The sample was rested on the rheometer for 10 min to minimise the stress caused during sample handling. A strain sweep (0.01 – 100 %) was performed at 20̊C, and a frequency of 1 Hz was selected to determine the viscoelastic region of paneer samples. A frequency sweep test was performed at 20̊C with a strain amplitude of 0.5 %, and the frequency ranged from 0.1 to 10 Hz. A temperature sweep was performed at a constant frequency of 1 Hz and a constant strain amplitude of 0.5 %. The temperature ranged from 20̊C to 80̊C at 5̊C/min using a Peltier heating system. All the measurements were taken in triplicate.

2.2.1.6. Confocal microscopy. The microstructure of CMP and CMMBP was examined using CLSM (Leica SP5 DM6000B scanning confocal microscope). Thin paneer slices were cut from the middle portion of the freshly prepared paneer. These slices were fixed onto a glass slide and were stained using Nile red fluorescent dye for labelling the fat phase and fast green FCF for staining the protein phase (Laursen et al., 2022). A cover slip was kept on the top of the cheese slide for 30 min to allow the dye to diffuse through the sample. An excess amount of dye was absorbed using Kim wipes (KIMTECH). The stained samples were kept under a light microscope under 63 × oil immersion objective lens, and the emission filters were set at 488 nm for Nile red and 633 nm for fast green. The CLSM micrographs were analysed using Image J software.

Similarly, mung bean curds were placed on the glass slide, stained, and looked through a confocal microscope, as mentioned above.

2.2.1.7. Texture profile analysis (TPA). A texture analyser (TA.XT. Plus

Texture analyser, UK) with a load cell capacity of 5 kg was used for determining the texture profile analysis of both CMP and CMMBP. The experiment was performed using a double compression test which generated a force–time graph to calculate the hardness, springiness, cohesiveness, and chewiness of the samples by modifying the method of Wan et al. (2021). The paneer samples were cut into cubes of 1 cm^3. A cylindrical probe (35 mm diameter) was used to compress the sample up to 70 % of its original length, and the pre-test speed, test speed and post-test speed of the probe were set to 0.5 mm/s, 1 mm/s and 5 mm/s, respectively. The reported data are an average of 5 replications per paneer type.

2.2.1.8. In vitro digestion. The *in vitro* digestion of the paneer samples was performed according to the method of Chian et al. (2019) as modified from Minekus et al. (2014). In vitro digestion experiments were done in triplicate for both CMP and CMMBP. Eight grams of paneer sample was crushed approx. 10 times using a mortar and pestle to achieve the chewed food-like texture and was then transferred into the two jacketed glass reactors. The samples were first incubated with a simulated salivary fluid having $1.25 \times 10^{-6\ \text{Kat}}$/mL of α-amylase (10025, Sigma Aldrich, Saint Louis, MO, USA) and the pH was maintained at 7.0 ± 0.1 for 2 min. This 2 min period represents the oral phase of digestion. It was followed by adding stimulated gastric fluid containing $1.33 \times 10^{-7\ \text{Kat}}$/mL protein of sample pepsin (P7000, Sigma Aldrich, Saint Louis, MO, USA), and the pH was adjusted to 3.0 ± 0.1. This marks the beginning of the gastric phase. 6–7 glass beads of 3–5 mm diameter were added to mimic the maceration of the paneer in the gastro-small intestinal tract. Samples were collected at 0, 10, 30 and 60 min of the gastric phase.

Small-intestinal digestion phase was initiated after 60 min of gastric digestion, with the addition of simulated intestinal fluid and pancreatin (P1750, Sigma Aldrich, Saint Louis, USA) at a 1:100 pancreatin to protein ratio. The pH during the intestinal phase was maintained at 7 ± 0.1 with continuous mixing. The samples during the intestinal phase were collected at 70, 120 and 180 min. 12 μL of Pepstatin A (ab141416 Abcam, Cambridge, UK) in methanol (0.5 mg/mL) was quickly added to every 1 mL gastric digesta taken, and 0.45 mL of SIGMAFAST protease inhibitor cocktail solution (S8820, Sigma Aldrich, USA) (1 tablet in 50 mL Milli-Q water) was mixed with every 1 mL of the small intestinal digesta to inactivate the digestive enzymes present in the digesta.

The digesta and enzyme inhibitor mixture was dispersed for 30 *sec* using a handheld disperser (5 mm diameter) (T 10 basic ULTRA-TURRAX Homogenizer, IKA, Germany) in an ice bath. The digests were then stored at −20°C for further analysis.

2.2.1.9. Ninhydrin reactive amino N. The ninhydrin reactive amino N was determined in the digests using the method of Chian et al. (2019). The frozen digested samples from *in vitro* digestion were thawed and centrifuged at 14,000 × *g* for 7 min (Eppendorf Minispin plus, Hamburg, Germany). The supernatant was filtered using a 0.45 μm PVDF syringe filter (Millex, Duluth, MN, USA). Ninhydrin reactive amino N (%) in the filtered supernatant was determined using ninhydrin reagent (N7285, Sigma Aldrich Pty Ltd, USA).

2.2.1.10. Confocal microscopy of the digested cheese samples. The digested paneer samples (0 min, 60 min, and 180 min) were stained using Nile red for the fat phase and Fast Green FCF for the protein phase using the method of Laursen et al. (2022). The samples were rested for 10 min and were examined using a scanning confocal microscope (Leica SP5 DM600B) under a 63 × oil immersion objective lens. An excitation wavelength of 480 nm was selected for Nile red, and 633 nm was selected for Fast green FCF.

2.2.1.11. SDS-PAGE. The hydrolysis of proteins in paneer during digestion was examined using reduced-Tricine-SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using the method performed by Chian et al. (2019). The gels were stained, fixed and washed before being scanned using a gel scanning densitometer (Molecular imager Gel doc XR, Bio-Rad laboratories Pty. Ltd, New Zealand).

2.2.2. Statistical data analysis

Microsoft Excel (Microsoft Corporation, U.S.A.) was used for calculating the mean ± SD (standard deviation) of all the obtained values and for graphically representing the results. The means were compared to determine the significant difference between the paneer samples by one-way Analysis of Variance (ANOVA) at $p < 0.05$ significance level using Minitab 19 software (Minitab Ltd., Coventry, U.K.).

3. Results and discussion

3.1. Selection of mung bean through preliminary experimentation

Various plant proteins, including soy, hemp, soy and mung bean, were tested for their ability to form paneer at different ratios, as mentioned in section 2.2.1. It was observed that all protein isolates could not form paneer-like products at or below the 50:50 ratio. The curds produced were very fine and could not be pressed into paneer. All protein isolates above a 70:30 ratio were able to partially replace cow milk to form hybrid paneer. Therefore, the 70:30 ratio was selected as the most suitable ratio to conduct other experiments. Mung bean protein was selected considering the factors of cost, availability, and its ability to form curd when acidified with citric acid. Mung bean protein was selected for this experiment due to its sustainable nature, low allergenicity, yield and gelation ability (**Supplementary data Table S1**). Due to the lack of articles on paneer incorporated with plant protein isolates, the literature on cheese was mainly used to discuss the results.

3.2. Physicochemical properties and microstructure of paneer

3.2.1. Composition of paneer

Table 1 summarises the moisture, protein, and fat composition of CMP and CMMBP. Moisture and protein contents of CMMBP were significantly higher, but the fat content was significantly lower than that of CMP. These results are in accordance with the moisture and protein contents of low-fat hybrid paneer incorporated with soy protein concentrate and soy protein isolate by Rinaldoni et al. (2014) and Kumar et al. (2011), respectively. These studies also showed an increase in moisture and protein contents with an increase in plant protein proportion. Moisture content is related to fat content, in which a higher fat content reduces the moisture-holding capacity of paneer (Kumar et al., 2011). In addition, Rinaldoni et al. (2014) concluded that it might be due to the hydrophilic nature of soy protein. Mung bean protein has a similar ratio of hydrophilic and hydrophobic amino acids to soy protein

Table 1
Composition, colour and textural parameters of paneer samples.

Component	Paneer samples	
	Cow Milk Paneer (%)	Hybrid CMMBP (%)
Moisture	52.15 ± 0.43^{a}	54.04 ± 0.42^{b}
Protein	19.2 ± 0.52^{a}	22.27 ± 1.01^{b}
Fat	20.84 ± 0.50^{a}	17.61 ± 0.56^{b}
L*	89.15 ± 1.10^{a}	86.37 ± 0.92^{b}
a*	-3.13 ± 0.14^{a}	-1.66 ± 0.07^{b}
b*	17.52 ± 0.90^{a}	18.12 ± 0.94^{a}
Hardness (N)	17.69 ± 0.58^{a}	12.81 ± 0.36^{b}
Cohesiveness	0.34 ± 0.02^{a}	0.24 ± 0.01^{b}
Chewiness (N)	3.03 ± 0.11^{a}	0.87 ± 0.36^{b}
Springiness	0.62 ± 0.07^{a}	0.34 ± 0.03^{b}

CMMBP, Cow milk-mung bean protein paneer.
Means (±SD) with different superscript letters among the two paneer samples are significantly different ($p < 0.05$).

(Brishti et al., 2017). Therefore, the higher moisture content in CMMBP might partly be due to the hydrophilic nature of mung bean protein. In conclusion, the results mentioned above may be influenced by the composition of ingredients used for the preparation of the two paneer types.

3.2.1.1. Colour analysis. Table 1 summarises the colour profile of paneer samples analysed according to CIELAB colour space. It shows a decrease in the L* value for CMMBP, indicating a slightly darker colour compared to CMP ($p < 0.05$). Similar results were obtained by Kumar et al. (2011) using soy protein isolate as a fat replacer in low-fat paneer. The decrease in the lightness of CMMBP can be due to a decrease in its fat content, as L* value is directly proportional to fat content because of the scattering of light by fat molecules (Kumar et al., 2011).

The negative a* value of the samples indicated that CMMBP has a lower greenness in contrast to CMP ($p < 0.05$), while the b* values of the samples indicated that CMMBP has a higher yellowness than CMP samples. However, the latter was not significantly different ($P > 0.05$). Similar a* and b* values of CMP was obtained by Sant'Ana et al. (2013) for minas fresh cheese made from cow and goat milk. In this study, the decreased greenness values in CMMBP may be due to a decrease in fat content by the addition of mung bean protein isolate, which could be due to the translucent appearance imparted by the removal of fat in low-fat cheese (Rinaldoni et al., 2014). The slightly higher b* values of CMMBP could be due to the increase in amine compounds which react with amino acids (Maillard reaction) to develop dark pigments (Akesowan, 2009). The slightly darker colour of CMMBP compared to CMP was also observed under the naked eye (**Supplementary data Figure S2**).

3.2.1.2. Rheological properties of paneer. The textural and body characteristics of cheese and how processing techniques and composition influence them can be determined by studying their rheological properties. The linear viscoelastic region for both paneer samples lied between 0.01——1.5 kPa. During the frequency sweeps, for both CMP and CMMBP, values of *G'* were significantly higher ($p < 0.05$) than the *G''* values (Fig. 1a). This is an indication of dominant elastic properties. This is a typical behaviour shown by solid viscoelastic materials (Moghiseh et al., 2021). The significant difference ($p < 0.05$) found between storage (elastic) and loss (viscous) moduli for both CMP and CMMBP samples and the slight variation with the frequency across the whole range of frequencies studied suggested a strong gel structure (Rinaldoni et al., 2014).

CMMBP showed higher G' and G'' values when compared to CMP (Fig. 1a). It may be due to the higher protein content of CMMBP through the incorporation of mung bean protein isolate. According to Farahani et al. (2014), there is an increase in viscoelastic properties with increased protein content for Siahmazgi cheese, an Iranian ewe's milk variety. Viscoelastic behaviour of cheese can be modified by changing its composition by incorporating fillers, i.e., plant proteins, fats and hydrocolloids that interact with casein protein (Ouyang et al., 2022).

The role of protein in the viscoelastic behaviour of cheese is different from fat and water. Viscoelastic properties, except for loss tangent, increase with higher protein content, which contrasts with the role of water and fat in influencing cheese texture (Farahani et al., 2014). This can result from an increasing number of different intra and intermolecular linkages within the cheese matrix with the increase in protein concentration. This may lead to enhancing the elastic nature of cheese, resulting in a more solid-like behaviour (Fox et al., 2017). When protein concentration increases, the unfolded protein molecules get closer to each other by attractive forces; thereby, the role of proteins in the cheese matrix becomes dominant over that of water and fat (Farahani et al., 2014). Conversely, Omrani Khiabanian et al. (2020) reported a reduction in storage modulus values, lowering the solid-like nature of the product with a higher concentration of plant protein substitution in cheese. The lowering of elastic properties of the cheese may be due to lower total solids, weakened bonds, and less proteolytic activity of protein components in the cheese (Omrani Khiabanian et al., 2020). The current study concluded that the replacement of milk with 30 % mung bean protein isolate has no adverse effect on the rheological properties of CMMBP when compared to the control CMP.

In the case of temperature sweeps (Fig. 1b), a decrease in the storage modulus (G') and loss modulus (G'') values in both paneer samples were observed with the increase in temperature from 20°C to 80°C. This indicates softening of the paneer with the increase in temperature. Higher G' values compared to G'' values were observed for both paneer samples, indicating a greater elastic nature than the viscous nature of the product throughout the tested temperature range. It is evident from

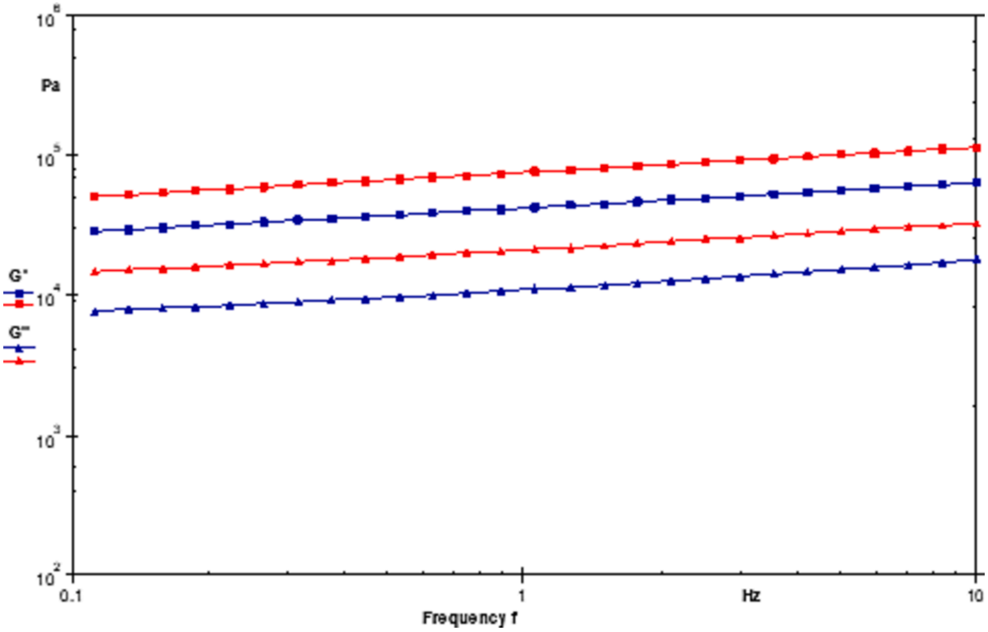

Fig. 1a. Changes in storage modulus (G′, squares) and loss modulus (G″, triangles) for cow milk paneer (blue) and hybrid cow milk-mung bean paneer (red) with increasing frequency (0.1——10) at 20 °C.

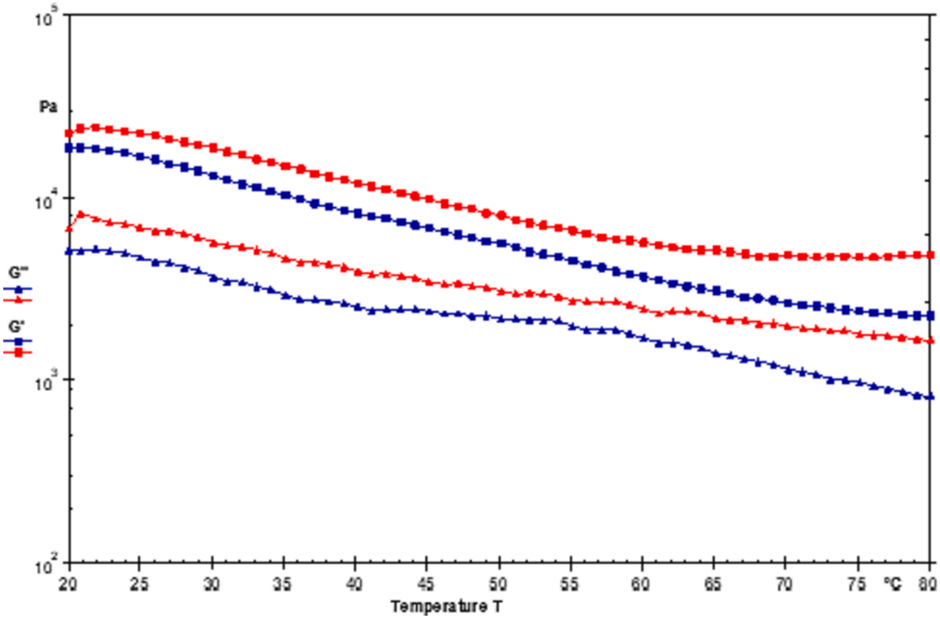

Fig. 1b. Changes in storage modulus (G′, squares) and loss modulus (G″, triangles) for cow milk paneer (blue) and hybrid cow milk-mung bean paneer (red) with temperature (20 – 80 °C) at a frequency of 0.1 Hz.

Fig. 1b that there was no crossover between storage modulus and loss modulus indicating a dominant solid-like nature of the paneer samples.

Tan δ (loss tangent) can be related to the relaxation of bonds within the cheese matrix. It can also indicate the flowability and melting properties of cheese (Mehfooz et al., 2021). *Tan δ* values<1 indicate the dominance of the elastic nature of the product over its viscous counterpart (Rinaldoni et al., 2014). According to Fig. 1c, tan *δ* values of both paneer samples were always <1, over the temperature range, indicating a dominant elastic nature of paneer. Lower values of tan *δ* indicate more solid-like properties. *Tan δ* also indicates a product's structural organisation. Products with highly organised structures show lower tan δ values.

Initially, at 20 °C, CMMBP showed a higher tan δ value which may be due to its higher moisture content (Fig. 1c). Farahani et al. (2014) showed that **tan** *δ* increased with increasing moisture content. CMMBP contained higher moisture content than CMP, according to moisture analysis data (Table 1). The behaviour of tan *δ* during the latter temperature towards 80̊C was opposite to that of initial temperatures, in which CMP recorded higher tan *δ* values than CMMBP. Previous studies have documented that increased fat content can lower the storage and loss modulus values while increasing the tan *δ* values (Farahani et al., 2014). This can be due to the role of fat as a lubricant and filler in the casein matrix, showing a more liquid-like behaviour in cheese texture (Dimitreli & Thomareis, 2008). Due to high temperatures in the latter part, moisture evaporation was possible from both samples. Then, the role of fat may have become more dominant. This may be the reason for higher tan *δ* values recorded for CMP than CMMBP.

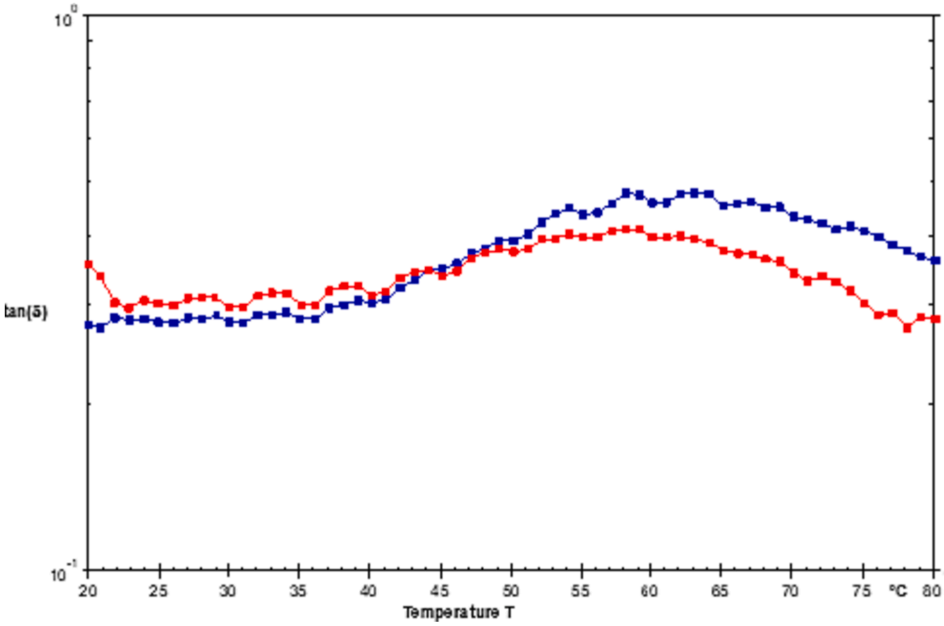

Fig. 1c. Changes in tan δ values for cow milk paneer (blue) and hybrid cow milk-mung bean paneer (red) with temperature (20 – 80 °C) at a frequency of 0.1 Hz.

3.2.1.3. Microstructural characteristics. Understanding the cheese microstructure is necessary for manufacturers, as various functional properties and their controlled development depend upon the location and interaction of multiple components within the cheese matrix. These constituents can be studied through their microstructure during various processing stages and storage. From Fig. 2A & B**)**, it can be observed that milk proteins formed the primary protein network. In the case of CMP, the fat globules were evenly distributed within the dense protein matrix, while in the case of CMMBP, protein aggregates of varying sizes could be observed within the casein matrix along with a few numbers of non-spherical pockets of fat with a higher degree of coalescence. Fig. 2 **(C)** shows the confocal image of the mung bean curd.

The formation of these aggregates in CMMBP can be explained as denaturation during heat treatment due to the unfolding of the tertiary and quaternary structure of both proteins. And then forming aggregates having high molecular weight due to the exposure of reactive functional groups causing interactions with other particles (Ben-Harb et al., 2018). Similar interactions have been observed by Mession et al. (2015), where denaturation causes exposure of free thiol-groups, which helps form disulphide linkages between β-lactoglobulin subunits or/and dissociated legumin subunits. Roesch and Corredig (2005) found that covalent bonds between soy protein subunits and β-lactoglobulin and α-lactalbumin could be created. Ben-Harb et al. (2018) observed that pea/milk mixed emulsion gels during heat/acid treatment showed similar protein aggregates of varying sizes. This aggregate size difference was linked to the highest risk of sample strain, indicating that the formed gels may be brittle. Similar bonds must have been created during the denaturation of mung bean protein with milk proteins in this study, which could explain the presence of these large aggregates. When two types of biopolymers, i.e. proteins-polysaccharides, proteins-proteins or polysaccharides-polysaccharides, are present in a system during gelation, they may interact with each other before or during gelation and thus affect the final characteristics of the gel network (Ersch et al., 2016).

However, Schmitt et al. (2019) concluded that adding plant protein to dairy protein decreases gel strength when compared to dairy protein alone. This is due to the formation of independent protein networks by dairy and plant protein sources that do not interact with each other. They also concluded that micellar casein did not form a protein network with plant protein during heating and formed independent networks.

3.3. *Texture profile analysis*

Cheese texture is an important indicator for evaluating the quality and functional properties of cheese and has a significant influence on customer choices and acceptance.

From Table 1, it can be observed that there was a significant decrease ($p < 0.05$) in the textural properties of CMMBP when compared to CMP. The lower hardness of CMMBP might be due to increased moisture content. Uprit and Mishra (2004) observed similar results on the textural properties of soy paneer made by blending soy milk and skimmed milk in different proportions. They concluded that the decrease in hardness might be due to an increase in the moisture content of the paneer and the higher moisture content retained by soy protein. Similarly, Rinaldoni et al. (2014) reported a decrease in hardness values in soft cheese with the incorporation of soy protein concentrate. The other TPA parameters such as cohesiveness, chewiness and springiness followed a similar trend like hardness, showing lower values for CMMBP (Table 1). The changes in the nature of the protein matrix and moisture distribution in the CMMBP samples may be responsible for the lower values of cohesiveness, chewiness and springiness. Omrani Khiabanian et al. (2020)

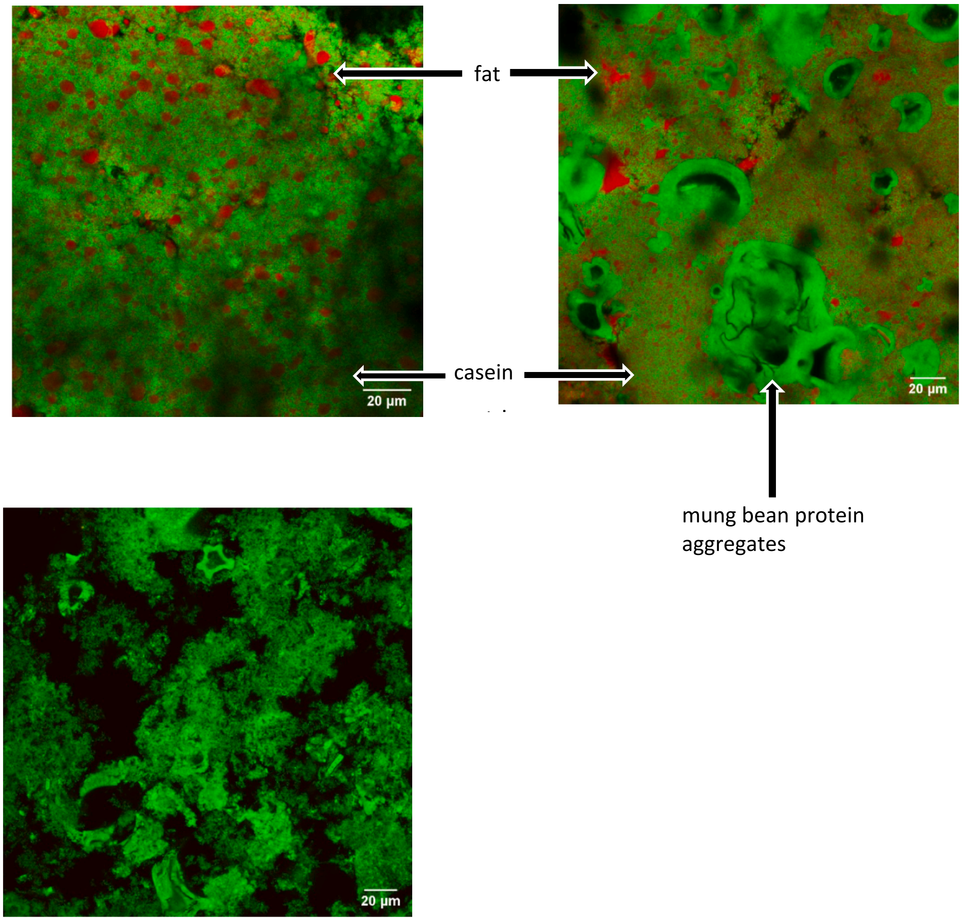

Fig. 2. Confocal images of (A) cow milk paneer, (B) hybrid cow milk-mung bean protein paneer (C) mung bean protein curd.

observed a decrease in textural properties with increased pea protein isolates in whey-less feta cheese. Both researchers reported a decrease in hardness values with increased plant protein concentration.

The textural parameters are determined by the structural arrangement of the components within the network, which is influenced by the composition and the processing methods employed. The lower textural properties of cheese may also be due to the presence of globular plant protein within the casein network, which prevents the formation of a compact protein network generally observed in coagulated dairy products, thus, weakening the texture (Omrani Khiabanian et al., 2020). The presence of protein aggregates of varying sizes within the casein network can also be confirmed by the confocal microscopy images (Fig. 2).

To conclude, high moisture content and the globular nature of mung bean protein present within the paneer matrices may have caused the reduction of textural properties in CMMBP.

Uprit and Mishra (2004) also suggested that hardness values are inversely proportional to fat content. However, CMMBP, despite having lower fat content, showed lower hardness values which might be due to a much stronger effect of mung bean protein aggregates within the milk protein network.

3.4. *In vitro digestion of paneer samples*

3.4.1. *Ninhydrin reactive amino N*

The Ninhydrin test was performed as a quantitative measurement of free amino N produced during simulated gastro-small intestinal digestion to assess the extent of proteolytic digestion of proteins in paneer. There was an increase in the release of free amino N with an increase in digestion time from 0 min to 180 min in both CMP and CMMBP (Fig. 3a). CMMBP showed a similar release pattern when compared to CMP, but a lower release of free amino N. Hydrolysis of both the paneer samples by pepsin during the gastric phase (0 min – 60 min) was limited, and no significant difference was observed between the two samples. Further, with the addition of a simulated small intestinal buffer during the small intestinal phase, a rapid increase in the release of free amino N was observed in the two digested paneer samples. However, the release of free amino N remained significantly lower ($p < 0.05$) in CMMBP than in CMP. It is understood from this result that the addition of mung bean protein has reduced the free amino N release in the case of CMMBP.

The results are in accordance with the generally accepted fact that animal proteins are better digested when compared to plant proteins (Kaur et al., 2022). Research on the digestion of matrices like yoghurt and cheese or similar matrices is limited compared to purified dairy proteins like whey and casein.

In this study, observations are in accordance with the previously reported results, which compared the static *in vitro* digestibility and dynamic *in vitro* digestibility of milk proteins with *in vivo* results (Kaur et al., 2022). The results obtained for static *in vitro* digestion of milk proteins showed a very minimal increase in the release of free amino acids (FAA) during the gastric phase compared to the small intestinal phase, which might be due to the inactivation of pepsin, by-product inhibition, or pepsin autolysis (Kaur et al., 2022). In the case of dynamic *in vitro* digestion, a slow increase in the release of free amino N was observed, which can be explained by the continuous addition of pepsin, as demanded by the dynamic protocol. Even though there was a gradual increase, the amount released was very low compared to the small intestinal phase.

A clear difference can be observed in the release of FAA at the beginning of the small intestinal phase with the addition of small intestinal enzymes. Protein hydrolysis has also been observed to be dependent on the type of matrix with rate of hydrolysis decreases with an increase in the solid nature of the matrices, i.e. liquid matrix (milk) > semi-solid matrices (yoghurt) > solid matrices (cheese) (Lamothe et al., 2017). Food microstructure is an important factor that influence the penetration of enzymes during gastro-intestinal digestion (Kaur et al., 2022).

Legume proteins are generally associated with low digestibility due to anti-nutritional factors, the structural arrangement of proteins, and complex formation due to the interaction of proteins with other seed components (Kaur et al., 2022). Their unique compact structure and 3-dimensional stability due to carbohydrate moiety can also lead to cause low digestibility.

Another reason for low digestibility could be the presence of β-sheet conformation in legume proteins, similar to β-Lactoglobulin present in bovine milk. This conformation is associated with resistance to denaturation during gastrointestinal digestion (Kaur et al., 2022). The digestibility of proteins greatly depends on the extent of denaturation. It is understood that proteolysis can only occur if the protease active site can bind to the specific stereochemistry of the protein to be digested (Lorieau et al., 2018). Savoie et al. (2005) studied the peptide release kinetics of casein, cod protein, soy protein, and gluten. They found that the peptides more resistant to digestion were rich in amino acids like proline and glutamic acid. Mung bean protein is rich in these two amino acids, which can be the reason for its low digestibility. Overall, the low protein digestibility of CMMBP may be attributed to the presence of anti-nutritional factors and complex formation due to the interaction of proteins with other seed components.

3.4.1.1. SDS-PAGE. The protein breakdown during *in vitro* gastro-small intestinal digestion of paneer was characterised using SDS-PAGE (Fig. 3b). With the increase in digestion time, there was an increase in

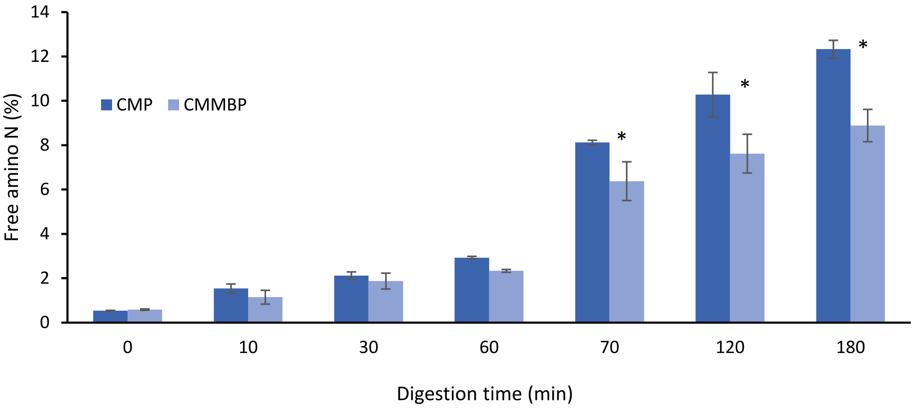

Fig. 3a. Free amino N released (%) during simulated gastric phase (0 – 60 min) and small intestinal phase (70 – 180 min) in cow milk paneer (CMP) and hybrid cow milk and mung bean paneer (CMMBP). Measurements were made in triplicate and the error bars represent the standard deviations. The bars having a * are significantly different among the two paneer samples at the same digestion time ($p < 0.05$).

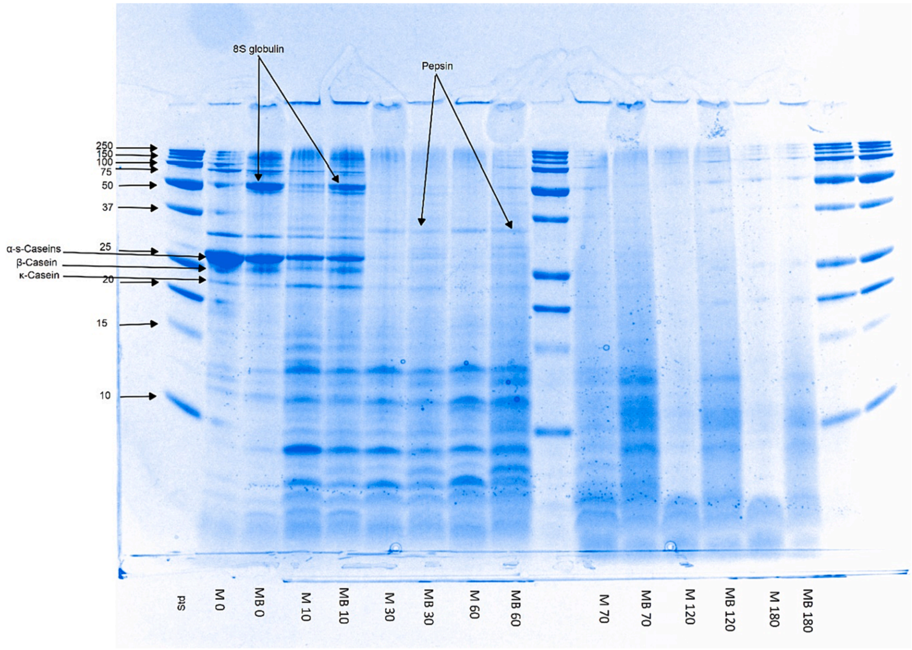

Fig. 3b. SDS-PAGE gels of hydrolysed paneer samples during gastric (0, 10, 30, 60 min) and small-intestinal (70, 120, 180 min) digestion phases for cow milk paneer and hybrid cow milk-mung bean paneer. M = cow milk paneer and MB = cow milk-mung bean paneer.

low molecular weight peptides in both the paneer samples, indicating protein hydrolysis. At 0 min, the bands for casein can be observed in both the paneer samples. After 10 min of gastric digestion, it can be noted that the casein bands have entirely disappeared in both the paneer samples. Fang et al. (2016) observed faster disintegration of casein during the gastric phase in Camembert cheese during *in vitro* digestion. Casein proteins are highly susceptible to proteolysis due to the preference of pepsin enzyme for more loosely structured polypeptides (Kaur et al., 2022).

Whey proteins like α-lactalbumin and β-lactoglobulin particularly β-lactoglobulin, are considered to be resistant to proteolysis by pepsin due to its well-defined globular intact structure, which blocks the enzymes from binding to the potential cleavage sites (Lorieau et al., 2018; Žolnere et al., 2019). Very low-intensity bands corresponding to α-lactalbumin and β-lactoglobulin were observed in the samples, as these are commonly released in whey, which is removed during paneer making.

Whey proteins can also form large aggregates with globulins present in mung bean protein through di-sulphide linkages. Minor interactions between whey and soy protein isolate were observed by McCann et al. (2018). During heating, whey proteins can unfold and can form di-sulphide linkages with other proteins as well as soy protein (McCann et al., 2018). The formation of similar aggregates with mung bean protein during digestion can prevent their separation in gels, causing low-intensity bands. Therefore, non-reducing SDS- PAGE may be done to identify if there is any aggregate formation between whey proteins and mung bean globulins.

In the case of CMP, after 10 min of small intestinal digestion, the peptides present were hydrolysed entirely by the action of enzymes. Fang et al. (2016) observed that the proteins were rapidly hydrolysed into smaller peptides and amino acids by adding intestinal juices and concluded that milk proteins are highly bio-accessible. While in the case of CMMBP, some bands were observed around 50 kDa. According to Kudre et al. (2013), the major bands observed for MBPI had 42, 48 and 54 kDa molecular weights. These bands were hydrolysed into peptides of smaller molecular weights within 10 min of gastric digestion. During the small intestinal phase, some bands were observed around 10–15 kDa, indicating enzyme-resistant peptides. This resistance to proteolysis in CMMBP might be due to anti-nutritional factors like trypsin inhibitors, polyphenols, and phytic acid (Kaur et al., 2022). The presence of trypsin inhibitors can affect the structure of globular protein and can prevent the digestive action of enzymes in the small intestine. Polyphenols present can form complexes with gastric enzymes causing inactivation thus, lowering protein digestibility (Kaur et al., 2022).

In the case of CMP, SDS-PAGE results showed that the protein bands disappeared with the increase in digestion time, while in the case of CMMBP, protein bands were still visible during the small intestinal phase. This can explain the higher amount of free amino N released in CMP during *in vitro* digestion compared to CMMBP, which is evident from the ninhydrin results.

Cheese texture plays a critical role in the rate of cheese disintegration during digestion, which may be related to their moisture content and protein network within cheese microstructure (Fang et al., 2016). Foods with lower initial hardness are known to disintegrate faster when compared to foods with higher hardness (Kong & Singh, 2009). In this experiment, CMMBP, due to its lower textural properties, might have disintegrated faster but showed lower rates of protein hydrolysis, which could be due to the globular nature of mung bean protein and the presence of enzyme-resistant peptides.

3.4.1.2. Confocal microscopy - microstructure of paneer digesta. During digestion, the food is partially disintegrated, allowing the trapped nutrients to mix with the digestive fluids (Kong & Singh, 2009). The effect of paneer breakdown and protein release was studied during its gastric and intestinal phase by adding enzymes like pepsin, pancreatin and bile salts. At 0 min, both paneer samples showed an initial breakdown in their protein matrix. Mixing of the paneer samples with gastric enzymes and electrolytes causes phase separation and physical instabilities of emulsions affecting protein digestion. It also results in the release of fat with the partial breakdown of protein structure. CMMBP showed higher amounts of fat droplets being released from the matrix (Fig. 4). In contrast, the fat release was lower in CMP. This could be attributed to the differences in the structure and material characteristics of both the paneer samples. The lower hardness and cohesiveness values of CMMBP may have led to its faster disintegration and fat release. The bigger size of fat droplets, as indicated by arrows (Fig. 4 **A & D**), can be explained

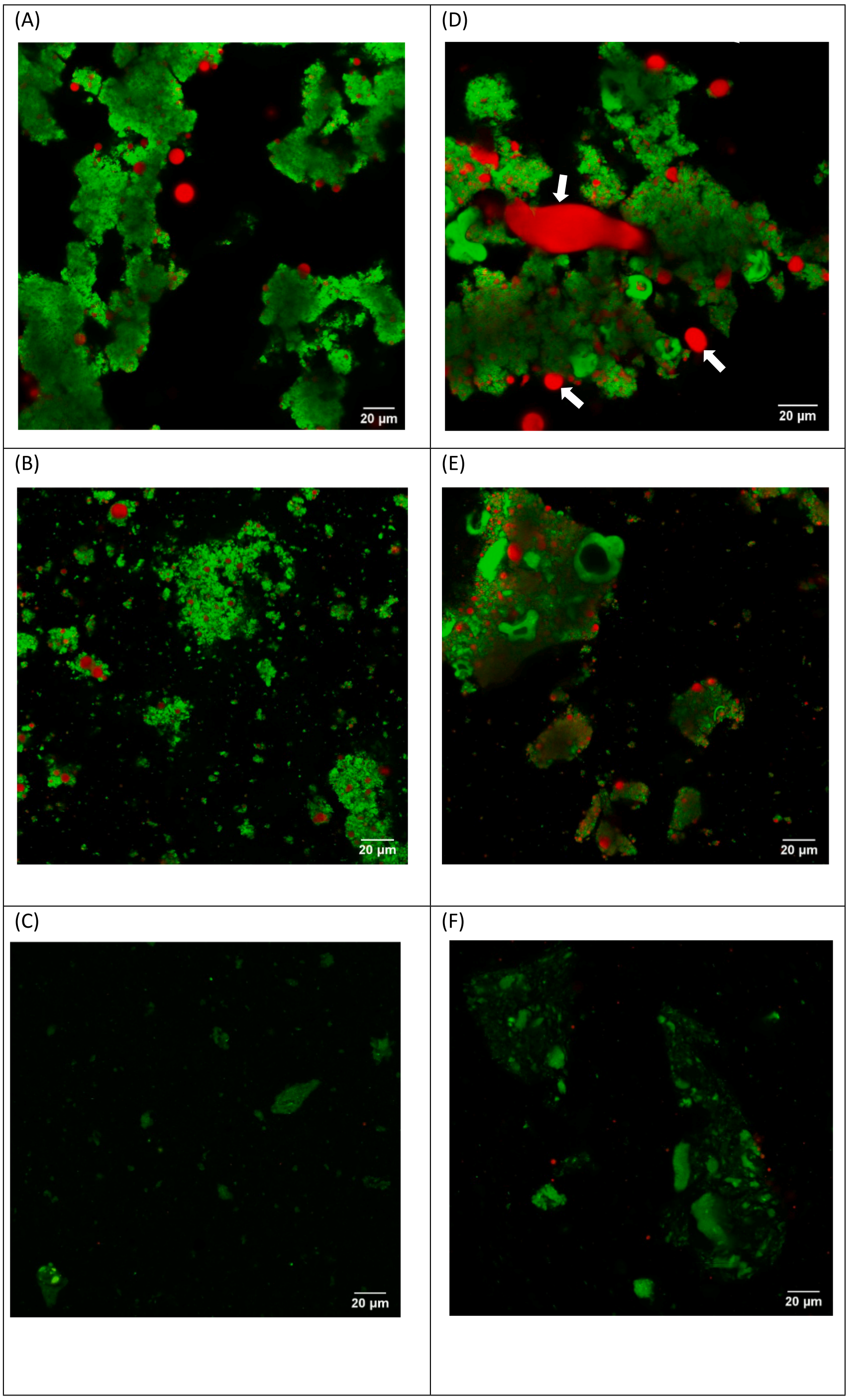

Fig. 4. Confocal microscopy images of cow milk paneer (CMP) after 0 min (A), 60 min (B), 180 min (C) and hybrid cow milk-mung bean paneer (CMMBP) after 0 min (D), 60 min (E) and 180 min (F) of *in vitro* digestion. Fats are shown in red, and proteins are shown in green.

due to fat globules retaining their native spherical shape due to re-emulsification by protein when released from the protein matrix; similar observations were reported by Žolnere et al. (2019). Results obtained by Guo et al. (2014) also showed that soft whey protein emulsion gels had a faster disintegration rate and fat droplets release when compared to hard whey protein emulsion gels during *in vitro* gastric digestion.

Significant breakdown of the protein matrix was observed after 60 min of gastric digestion in both the paneer samples (Fig. 4 **B & E**). After 180 min of digestion, the protein matrix of the CMP was completely disintegrated with few small protein structures remaining when compared to CMMBP, which showed a huge number of larger protein aggregates. The disintegration of the paneer matrix was higher during the intestinal phase than that of gastric phase. In the case of both paneer samples, the size of fat globules was reduced during the intestinal phase. The results were similar to the ones obtained by Žolnere et al. (2019).

The increase in the release of α-amino N in both paneer samples during the small intestinal phase, which is evident from the results of ninhydrin analysis in section 3.4.1, can be due to the near-complete disintegration of the protein matrix as shown in Fig. 4 (C&F).

4. Conclusions

This study attempted to develop hybrid paneer by partially replacing milk with mung bean protein isolate. The results revealed that the paneer made by replacing 30 % cow milk with mung bean protein isolate had higher protein and moisture levels than cow milk paneer, which can be explained due to high moisture retention by mung bean protein. Confocal microscopy results showed the presence of protein aggregates of varying sizes within the microstructure of paneer containing MBPI, thus preventing the formation of a compact protein network. Texture profile analysis of the paneer samples showed that textural properties like hardness, cohesiveness, springiness, and chewiness values reduced significantly in CMMBP compared to CMP, possibly due to its high moisture content and the presence of protein aggregates within the paneer microstructure leading to a weak structure. Furthermore, *in vitro* gastro-small intestinal digestion experiments performed on the samples showed lower protein digestibility of CMMBP. The confocal microstructure of the paneer digesta also showed lower disintegration in CMMBP after 180 min of digestion. Further studies need to be done to improve the textural properties of CMMBP by adding calcium chloride, which is known to enhance the formation of protein cross-linkages. In addition, different hydrocolloids, such as carboxymethyl cellulose and pre-gelatinised starch, may improve the textural and sensory properties of CMMBP. In addition to the research on improving the quality of plant protein-incorporated paneer, the health benefits associated with the possible presence of bioactive peptides can also be explored for this novel food product.

CRediT authorship contribution statement

Shince Tojan: Investigation, Writing – original draft, Formal analysis. **Lovedeep Kaur:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Jaspreet Singh:** Writing – review & editing.

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.

Data availability

Data will be made available on request.

Acknowledgements

The authors thank the School of Food and Advanced Technology (Massey University) for funding this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.137434.

References

Akesowan, A. (2009). Influence of soy protein isolate on physical and sensory properties of ice cream. *Thai Journal of Agricultural Science, 42*(1), 1–6.

Aoac. (2016). *Official Methods of Analysis of AOAC International; AOAC International: Gaithersburg*. USA: MD.

Ben-Harb, S., Panouillé, M., Huc-Mathis, D., Moulin, G., Saint-Eve, A., Irlinger, F., … Souchon, I. (2018). The rheological and microstructural properties of pea, milk, mixed pea/milk gels and gelled emulsions designed by thermal, acid, and enzyme treatments. *Food Hydrocolloids, 77*, 75–84.

Boland, M. J., Rae, A., Vereijken, J., Meuwissen, M., Fischer, A., van Boekel, M., … Hendriks, W. (2013). The future supply of animal-derived protein for human consumption. *Trends in Food Science & Technology, 29*(1), 62–73.

Brishti, F. H., Zarei, M., Muhammad, S. K. S., Ismail-Fitry, M. R., Shukri, R., & N., s.. (2017). Evaluation of the functional properties of mung bean protein isolate for development of textured vegetable protein. *International Food Research Journal, 24.4 (1595–1605)*.

Cai, R., McCurdy, A., & Baik, B. K. (2002). Textural property of 6 legume curds in relation to their protein constituents. *Journal of Food Science, 67*(5), 1725–1730.

Chian, F. M., Kaur, L., Oey, I., Astruc, T., Hodgkinson, S., & Boland, M. (2019). Effect of Pulsed Electric Fields (PEF) on the ultrastructure and in vitro protein digestibility of bovine longissimus thoracis. *LWT, 103*, 253–259.

Ersch, C., Meinders, M. B., Bouwman, W. G., Nieuwland, M., van der Linden, E., Venema, P., & Martin, A. H. (2016). Microstructure and rheology of globular protein gels in the presence of gelatin. *Food Hydrocolloids, 55*, 34–46.

Fang, X., Rioux, L.-E., Labrie, S., & Turgeon, S. L. (2016). Commercial cheeses with different texture have different disintegration and protein/peptide release rates during simulated in vitro digestion. *International Dairy Journal, 56*, 169–178.

Farahani, G., Ezzatpanah, H., & Abbasi, S. (2014). Characterization of Siahmazgi cheese, an Iranian ewe's milk variety: Assessment of physico-chemical, textural and rheological specifications during ripening. *LWT-Food Science and Technology, 58*(2), 335–342.

Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. (2017). *Fundamentals of cheese science*. Springer.

Guo, Q., Ye, A., Lad, M., Dalgleish, D., & Singh, H. (2014). Effect of gel structure on the gastric digestion of whey protein emulsion gels [10.1039/C3SM52758A]. *Soft Matter, 10*(8), 1214–1223.

Hosseini-Parvar, S. H., Matia-Merino, L., & Golding, M. (2015). Effect of basil seed gum (BSG) on textural, rheological and microstructural properties of model processed cheese. *Food Hydrocolloids, 43*, 557–567.

Imarc. (2023). *Paneer Market: Global Industry Trends*. Opportunity and Forecast 2023–2028.

Kaur, L., Mao, B., Beniwal, A. S., Kaur, R., Chian, F. M., & Singh, J. (2022). Alternative Proteins Vs Animal Proteins: The Influence of Structure and Processing on Their Gastro-Small Intestinal Digestion. *Trends in Food Science and Technology, 122*, 275–286.

Kong, F., & Singh, R. P. (2009). Modes of Disintegration of Solid Foods in Simulated Gastric Environment. *Food Biophysics, 4*(3), 180–190.

Kudre, T. G., Benjakul, S., & Kishimura, H. (2013). Comparative study on chemical compositions and properties of protein isolates from mung bean, black bean and bambara groundnut. *Journal of the Science of Food and Agriculture, 93*(10), 2429–2436.

Kumar Rai, D., Niranjan, K., & Bhat, Z. F. (2014). Paneer—An Indian soft cheese variant: A review. *Journal of Food Science and Technology, 51*(5), 821–831.

Kumar, S., Balasubramanian, S., Biswas, A., Chatli, M., Devatkal, S., & Sahoo, J. (2011). Efficacy of soy protein isolate as a fat replacer on physico-chemical and sensory characteristics of low-fat paneer. *Journal of Food Science and Technology, 48*(4), 498–501.

Lamothe, S., Rémillard, N., Tremblay, J., & Britten, M. (2017). Influence of dairy matrices on nutrient release in a simulated gastrointestinal environment. *Food Research International, 92*, 138–146.

Lorieau, L., Halabi, A., Ligneul, A., Hazart, E., Dupont, D., & Floury, J. (2018). Impact of the dairy product structure and protein nature on the proteolysis and amino acid bioaccessiblity during in vitro digestion. *Food Hydrocolloids, 82*, 399–411.

McCann, T. H., Guyon, L., Fischer, P., & Day, L. (2018). Rheological properties and microstructure of soy-whey protein. *Food Hydrocolloids, 82*, 434–441.

Mehfooz, T., Mohsin Ali, T., Ahsan, M., Abdullah, S., & Hasnain, A. (2021). Use of hydroxypropylated barley starch as partial casein replacer in imitation mozzarella cheese. *Journal of Food Processing and Preservation, 45*, e16094.

Mession, J.-L., Chihi, M. L., Sok, N., & Saurel, R. (2015). Effect of globular pea proteins fractionation on their heat-induced aggregation and acid cold-set gelation. *Food Hydrocolloids, 46*, 233–243.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., … Dupont, D. (2014). A standardised static in vitro digestion method suitable for food–an international consensus. *Food & Function, 5*(6), 1113–1124.

Moghiseh, N., Arianfar, A., Salehi, E. A., & Rafe, A. (2021). Effect of inulin/kefiran mixture on the rheological and structural properties of mozzarella cheese. *International Journal of Biological Macromolecules, 191*, 1079–1086.

Motoyama, T, and S. Ashida. 2014. Mung bean protein gel composition and cheese-like food. Chinese Patent. CN105077227A, filed May 15, 2014 and issued November 25, 2015.

Omrani Khiabanian, N., Motamedzadegan, A., Naghizadeh Raisi, S., & Alimi, M. (2020). Chemical, textural, rheological, and sensorial properties of wheyless feta cheese as influenced by replacement of milk protein concentrate with pea protein isolate. *Journal of Texture Studies, 51*(3), 488–500.

Ouyang, H., Kilcawley, K. N., Miao, S., Fenelon, M., Kelly, A., & Sheehan, J. J. (2022). Exploring the potential of polysaccharides or plant proteins as structuring agents to design cheeses with sensory properties focused toward consumers in East and Southeast Asia: A review. *Critical Reviews in Food Science and Nutrition, 62*(16), 4342–4355. https://doi.org/10.1080/10408398.2021.1874869

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.

Roesch, R. R., & Corredig, M. (2005). Heat-induced soy− whey proteins interactions: Formation of soluble and insoluble protein complexes. *Journal of Agricultural and Food Chemistry, 53*(9), 3476–3482.

Savoie, L., Agudelo, R. A., Gauthier, S. F., Marin, J., & Pouliot, Y. (2005). In vitro determination of the release kinetics of peptides and free amino acids during the digestion of food proteins. *Journal of AOAC International, 88*(3), 935–948.

Schmitt, C., Silva, J. V. C., Amagliani, L., Chassenieux, C., & Nicolai, T. (2019). Heat-induced and acid-induced gelation of dairy/plant protein dispersions and emulsions. *Current Opinion in Food Science, 27*, 43–48.

Uprit, S., & Mishra, H. (2004). Instrumental textural profile analysis of soy fortified pressed chilled acid coagulated curd (Paneer). *International Journal of Food Properties, 7*(3), 367–378.

Verma, A. K., Kumar, S., Das, M., & Dwivedi, P. D. (2013). A Comprehensive Review of Legume Allergy. *Clinical Reviews in Allergy & Immunology, 45*(1), 30–46.

Žolnere, K., Arnold, M., Hull, B., & Everett, D. W. (2019). Cheese proteolysis and matrix disintegration during in vitro digestion. *Food Structure, 21*, Article 100114.

Laursen, A. K., Rovers, T. A. M., Ipsen, R., & Ahrné, L. (2022). Texture and microstructure of heat and acid induced gels from buffalo and cow milk: Effect of thermal treatment and fat content of milk. *International Dairy Journal, 129*, Article 105299. https://doi.org/10.1016/j.idairyj.2021.105299

Wan, Z., Misra, N. N., Li, G., & Keener, K. M. (2021). High voltage atmospheric cold plasma treatment of Listeria innocua and Escherichia coli K-12 on Queso Fresco (fresh cheese). *LWT - Food Science and Technology, 146*, Article 111406. https://doi.org/10.1016/j.lwt.2021.111406