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Methodology and development of a high-protein plant-based cheese alternative

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ABSTRACT

Animal-based food products, such as meat and dairy, contribute the most to greenhouse gas emissions in the food sector. This, coupled with the demonstrably worsening climate crisis, means that there needs to be a shift to more sustainable alternatives in the form of plant-based foods. In particular, the plant-based cheese alternative industry is relevant, as the products lack critical functionalities and nutrition compared to their dairy-based counterparts. Waxy starch, plant-protein isolate, and coconut oil were combined to create a novel high-protein (18% w/w) plant-based cheese alternative. We determined that when using native waxy starch, we can enhance its existing viscoelastic properties by modulating gelatinization through adding plant protein and fat. Texture profile analysis indicated that the cheese analogues could reach hardness levels of 15–90N, which allowed samples to be tailored to a broader range of dairy products. We determined that plant proteins and fat can behave as particulate fillers, enhance network strength, and create strategic junction points during starch retrogradation. The degree of melt and stretch of the high-protein plant-based analogues were 2–3 times greater than those observed for commercial plant-based cheese alternatives and significantly more similar to dairy cheese. The rheological melting kinetics saw that the high-protein plant-based cheese alternative displayed more viscous properties with increasing temperature. Tan δ (G''/G') at 80 °C was used as an indicator for sample meltability where, values ≥ 1 indicate better melt and more viscous systems. The high-protein plant-based cheese alternative reached Tan δ values upwards to 0.7, whereas commercial plant-based cheese alternatives only reached tan δ values around 0.1. Ultimately, the novel high-protein plant-based cheese alternative demonstrates the use of simple ingredients to form complex food systems.

1. Introduction

For every kilogram of dairy cheese, 24 kg of CO_2 is produced; for perspective, the emissions are equivalent to those generated when driving 150 km (Ritchie et al., 2022). The process of traditional cheese-making has a significant impact on the environment. The ethical issues related to the milking of cows, their land usage, and the direct production of methane further compound the problem. Additionally, transportation and processing contribute to the overall unsustainability of this food practice. To combat the rising environmental burden, there needs to be a shift to more sustainable food alternatives.

The plant-based cheese alternative industry is one of the fastest-growing plant-based food sectors. In the past two years, the US sales for plant-based cheese alternatives have increased by 70%, with reported US sales of 270 million in 2020 (GFI, 2021). The rise in popularity of plant-based cheese alternatives can be attributed to several factors, including a growing number of individuals with dairy allergies, societal acceptance, and competitive pricing. Despite the impressive numbers, there is still plenty of room for further growth in this market.

The main categories in the plant-based cheese alternative sector include nut-based products, plant-based milk alternatives, and starch and oil-based cheese (Saraco, 2021; Short et al., 2021). The ingredients used in these products have a significantly lower environmental impact, creating less than 2.5 kg of CO_2/kg of product (Ritchie et al., 2022).

The technology surrounding plant-based cheese alternatives was recently summarized by Grossmann and McClements (2021). They identified two protein-based categories. 1) Protein-based dilute dispersion where plant proteins are extracted from a crop and suspended in used in the formulation, and the protein creates the continuous phase (Grossmann and McClements, 2021). 2) Protein-based concentrated dispersion, where a plant-based paste from food sources such as nuts is used, and protein creates the continuous phase (Grossmann and

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McClements, 2021). These cheeses provide increased nutritional properties but lack functionality in ways such as melt and stretch.

Plant proteins used in plant-based milk and concentrate dispersions comprise of albumin and globulins (Singhal et al., 2016). There are notable differences between the structures of plant-based milk and dairy cheese, with protein casein being one of the main distinguishing factors. Dairy cheese is made by acidifying, coagulating, and ripening casein, resulting in cheese formation. Although plant-based milk and pastes undergo similar initial processes like acidification, aggregation, and aging, the protein structure during heating (i.e., the melting processes) differs from dairy cheese. In dairy cheese, as it is heated, the cheese network starts to soften due to the melting of milk fat around 40 °C; as heating continues, the casein network contracts due to increased hydrophobic interaction (Lamichhane et al., 2018; Lucey et al., 2003). The contraction of the network leads to decreased contact of casein molecules, which creates overall network weakening and thus continued softening or melting of the cheese(Lamichhane et al., 2018; Lucey et al., 2003). For plant-based proteins, heating has the opposite effect and, instead, causes denaturation and formation of irreversible gels that create more rigid structures.

The exception to this case is, however, prolamin proteins; Mattice and Marangoni (Mattice & Marangoni, 2020a, 2020b) discovered that by using zein protein from corn with additional starches and gums, the viscoelastic properties of zein could create similar melting properties to cheese. Prolamins, when plasticized, have a glass transition temperature of around 40 °C; at this temperature, crosslinking of zein protein dominates, and the hydrophobic mass can stretch and become fluid (Mattice & Marangoni, 2020a, 2020b). The limitation of this method is that zein is not a complete protein, and its limited fraction in existing crops makes the product expensive to produce.

Starch and oil-based cheese products tend to have the best melting properties and are therefore extensively marketed for foods such as pizza, nachos, and grilled cheese, as they more closely fit the desired sensory profile (Grasso et al., 2021). For applications in plant-based cheese, blends of high amylopectin starches are commonly selected. The amylopectin provides increased swelling capacity, viscoelasticity and contributes to gel reversibility which results in the system displaying melting or softening properties. In addition, amylose-containing starches or modified starches are also incorporated to provide additional structure to the network. However, the perceived melting or softening observed from current commercial plant-based cheese alternatives comes with a cost to the nutritional properties. Limited to no protein is present in commercial plant-based cheese products (Fresán and Rippin, 2021; Grasso et al., 2021). In comparison, dairy-based cheeses have approximately 3–7g of protein per serving. For those on a plant-based diet, protein is of utmost importance, making it essential that plant-based products have protein equality.

We believe that strategically incorporating albumin and globulin plant proteins into a starch-based cheese system is a superior method for providing better nutrition paired with improved functionality. In previous research from our laboratory, rapidly swelling waxy maize starch and pea protein were combined to create a supporting network (Dobson et al., 2022). The processes involved no heat; thus, protein aggregation and denaturation were not an issue; instead, the system was treated as a particle-filled network (Dobson et al., 2022). We approached the development of a high-protein plant-based cheese in the same way. This research will examine a cheese formulation that was created using waxy starch, fat and incorporating plant protein to create a high-protein plant-based cheese.

The study aims to qualitatively and quantitatively evaluate commercial dairy and plant-based cheese alternatives through a series of analytical methods. Through this process, the high-protein plant-based cheese formula will be compared with existing commercial samples. Ultimately, this research will provide valuable insights into cheese evaluation and introduce an innovative, high-protein plant-based cheese option.

2. Materials and methods

2.1. Materials

Commercial cheeses were purchased from a local grocery store; Cracker Barrel Mild Cheddar (Canadian Cheese Corporation, Toronto, ON, CA). Kraft Singles (KraftHeinz Canada, Toronto, ON, CA). Daiya Cheddar flavour slices (Daiya Foods INC., Burnaby, BC). Earth Island®, non-GMO Cheddar Style Slices (Product of Greece. Manufactured for Earth Island®, Chatsworth, CA). Violife Cheddar Style Slices alternative to cheese (Produced in Greece By: ARIVIA S.A. Block 31 Industrial Area of Sindos, Thessaloniki, Greece). Sheese® Vegan, Mature Cheddar style Slices, Non-dairy Simulated Cheese Product (Made By: Rothesay Isle of Bute, Scotland, U.K.) For the high-protein plant-based cheese formulation, Fava protein isolate and waxy maize starch were supplied by a commercial company. Coconut Oil, Refined, Organic, non-GMO. (Nutiva® Nurture Vitality™, Product of Philippines. Manufactured for: Nutiva®, Richmond, CA) was purchased from local retailers. Citric acid was purchased from Sigma Aldrich, (Mississauga, ON, Canada)

2.2. Cheese analysis methods

2.2.1. Texture profile analysis

Texture profile analysis (TPA) is a standard technique used to obtain sensory characteristics of food. TPA mimics the first two bites of chewing by compressing the food to a desired level of deformation. The test was used to determine the sample hardness.

Hardness: The peak force of the first compression.

To analyze the cheese products, samples were prepared using a cylindrical die cutter with a 20 mm diameter, then trimmed to 10 mm in height. For pre-sliced commercial samples, the die cutter was used to cut samples, which were then stacked to reach 10 mm in height. All samples were kept at 5 °C and analyzed within 1–5min of being cut. The sample disks were analyzed using a TA.XT2 texture analyzer (Stable Microsystems, Texture Technologies Corp. Scarsdale, NY, USA) fitted with a 75 mm cylindrical plate and 30 kg load cell. The samples were compressed to 50% of their original height at a crosshead speed of 1.00 mm/s with 5sec rest between compressions. The data was recorded in newtons and analyzed using Exponent software.

2.2.2. Disk melt test (modified Schreiber test)

The meltability of the cheese was measured using a modified Schreiber test. Samples were cut with a cylindrical 20 mm die cutter, then trimmed to be 10 mm in height. Samples in sliced form were cut to be the same 20 mm diameter and stacked to be 10 mm in height. The samples were kept at 5 °C. A paper template 90 mm in diameter was printed with increasing concentric circles every 5 mm, and lines at 45° angles (Figure S1) were placed at the bottom of each Petri dish facing up. The sample was then placed on top of the template (Supplementary Fig. 1), covered with the corresponding glass top, and placed in the refrigerator at 5 °C for 10 min. The samples were then transferred to an oven preheated to 232 °C (450 °F) for 5 min. The samples were removed and allowed to cool before the diameter of the spread at four different angles was taken. The measurement average was used to calculate the meltability by determining the percentage increase in diameter from the initial 20 mm.

2.2.3. Oil loss

Oil loss for the samples was measured based on the saturation of Schreiber disk paper (Supplementary Fig. 1) that occurred during the Disk melt test outlined in section 2.2.2. The degree of saturation was visually observed by identifying a change in translucency of paper. The oil saturation was measured at eight different points on the melt disk paper which were then averaged and expressed as a percentage of saturated area.

2.2.4. Rheometer temperature sweep

Oscillatory shear strain tests and temperature sweeps were performed using a rotational rheometer (MRC 302, Anton Paar, Graz, Austria) equipped with a 20 mm parallel plate geometry (PP20/S). To avoid slipping, the top and bottom plates were affixed with 40 and 600-grit sandpaper, respectively, and a small amount of super glue was used to adhere the sample. The samples were less than 3 mm in height and were compressed between the plates with an axial force not exceeding 5 N. The normal force was then reduced to 0.25 N and held for 3 min to allow the sample to relax. Peltier plates and a forced air hood (Anton Paar, Graz, Austria) were used to control the temperature.

Amplitude sweeps were first performed at 5 °C, 25 °C and 50 °C on commercial Kraft Single slices to determine the linear viscoelastic region (LVR). The sweep was performed at a logarithmic rate from 0.01 to 200% strain at a constant frequency of 1Hz. A frequency sweep from 1 to 10Hz was then carried at 0.1% strain which was within the LVR.

To investigate the melting profile of the cheeses, A temperature sweep from 5 to 80 °C at a rate of 5 °C per min was carried out at 0.1% strain, at a frequency of 1Hz with a constant normal force of 0.25N to adjust for sample melting. The variables obtained for all tests were storage modulus (G’), loss modulus (G”) and Tan δ (G”/G’). The data was analyzed using RheoCompass Software. For the purpose of this investigation melt or meltability will be referred to when describing the exposure of dairy or plant-based cheese to heat and the resulting visual or structural changes.

2.2.5. Axial pull

The extensibility/stretch of the cheeses was measured using a rotational rheometer (MRC 302, Anton Paar, Graz, Austria) with Peltier plates and a forced air hood (Anton Paar, Graz, Austria) used for temperature control. The rheometer was fitted with a 20 mm parallel plate geometry (PP20/S) and preheated to 80 °C. To avoid slipping, the top and bottom plates were affixed with 40 and 600-grit sandpaper, respectively, and a small amount of super glue was used to adhere the sample. 5 mm samples were used and compressed between the plates with an axial force not exceeding 5 N. The normal force was then reduced to 0.25 N. The samples were held for 6 min at 80 °C with a constant 0.1% strain and applied normal force 0.25N. The applied force ensured continuous contact with the sample during melting, but the gap decrease was limited to a height of 3 mm. After heating, an axial pull was performed where the top parallel plate geometry moved upwards at 1500um/s. The Normal force (N) and Gap (mm) were recorded during the pull using RheoCompass Software. Additionally, a video recording of the axial pull was done using the camera of an iPhone XS (Apple Inc.). The gap size of the instrument was recorded in the same frame as the sample stretch, and the gap at which the sample broke was used as the breakpoint. Total stretch was calculated using ((Eq. (1))

$$Stretch(mm) = Breakpoint\ (mm) - Starting\ gap\ after\ heating\ (mm) \qquad \text{(Eq. 1)}$$

2.3. High-protein plant-based cheese methods

2.3.1. Formulation

High-protein plant-based cheese was created using a formulation containing 18%w/w protein ingredient, 21%w/w fat in the form of coconut oil, 12%w/w waxy corn starch and 49%w/w water. The mixture was adjusted to pH 5.5 using a 1M citric acid solution. Fava protein isolate was selected as the main protein in the formulation as it had the highest protein purity of 91% what could be obtained commercially.

2.3.2. Mixing method

A 5%w/v protein solution was first formed using the protein ingredient and aqueous portion. The solution was combined using a stir plate set to 400 rpm for 10min to allow protein dispersion. The coconut oil was then heated via microwave to become liquid and combined with the protein solution under homogenization at 20,000 rpm using a hand homogenizer. The emulsion was then transferred into a Thermomix TM6 heated blender. The emulsion was mixed at 200 rpm while the remaining dry protein and dry starch fraction were added until all dry powder was gone and no clumps were present. 1M citric acid solution was also added to bring the pH of the cheese to 5.5.

The mixture then underwent heated mixing, which involved the following steps:

1) Temperate ramp from 40°C to 80 °C with mixing at 200 rpm
2) Temperature hold at 80 °C with speed ramp to 750 rpm
3) Temperature hold at 80 °C with mixing at 75 rpm
4) Temperature hold at 80 °C with speed ramp up to 750 rpm
5) Temperature hold at 80 °C

Samples were taken at different time points, Identified as Time 1-Time 7. The cheese was then refrigerated for 24h prior to testing.

Note: Multiple heating methods were tested, however the one outlined above was determined the most reproducible and easy to perform under the desired time periods.

2.4. Statistical analysis

All samples were prepared in at least duplicate. GraphPad Prism 9.0 (GraphPad Software, SanDiego, CA, USA) was used for statistical analysis of all data. Significance between samples ($P < 0.05$) was determined using one-way ANOVA followed by Tukey’s Multiple Comparison Test.

3. Results

3.1. Compositional evaluation of commercial dairy and plant-based cheese

Commercial dairy and plant-based cheese alternatives were investigated to establish a reference and identify areas that required improvement. The composition of the cheeses can be seen in Table 1. Conventional dairy cheeses possess significantly greater amounts of protein than all commercial plant-based samples. However, the amount of protein between the cheddar and Kraft Single is different, with cheddar reaching 23% protein and a Kraft Single reaching 16% protein. The difference can be attributed to the cheese-making process where mild cheddar uses traditional cheese making, and the block comprises solely milk protein. In comparison, processed cheese includes a portion of natural dairy cheese and additional ingredients that act as extenders

Table 1
Composition of commercial dairy cheeses, commercial plant-based cheese alternatives, and novel high-protein plant-based cheese alternative. Values for commercial samples were obtained from the nutritional labels on the packaging of samples, and values for the novel formulation were calculated from the proportion of ingredients used.

Samples	Protein content of cheese (%)	Fat (%)	Total starch/ Carbohydrate (%)
Dairy			
Kraft Single	16	21	11
Medium Cheddar	23	37	0
Plant-Based			
Daiya Cheddar	0	23	18
Violife Cheddar	0	23	20
EarthIsland Cheddar	0	23	20
Sheese Cheddar	0.3	24	20
High-Protein Plant-Based			
Novel Formulation	16	21	12

and emulsifiers to stabilize the systems(Caric et al., 1985). This is reflected in the carbohydrate content of the Kraft Single, which makes up 11% of the product, whereas conventional cheddar contains no additional carbohydrates. Plant-based cheese alternatives are similar to processed cheeses in that they also contain carbohydrates. However, the proportion of carbohydrates in plant-based cheese alternatives is much greater, comprising 18–20% of the product, and the cheeses have little to no protein in return. Only one of the commercial samples, Sheese, contained a trivial 0.3% protein. The limited protein combined with significant carbohydrate content indicates that current commercial plant-based cheese alternatives cannot compare nutritionally to conventional dairy cheese.

High-protein plant-based cheese alternative was developed containing 16% protein, 21% fat and 12% carbohydrate, as listed in Table 1. The composition aimed to reach protein levels as close as possible to conventional dairy cheese. The formulation utilized fava protein isolate, which contained 91% protein purity. The high-protein purity ensures that additional carbohydrates in the protein ingredient remain minimal, not to influence the behaviour of the novel cheese system. The novel formulation presents a protein value 85% greater than the current commercial plant-based cheese products and similar to that of the dairy-based Kraft Single. The fat and starch contents are also more similar to dairy cheese than the current commercial plant-based products. The novel formulation has a superior nutritional composition than all commercial plant-based and is competitive with current dairy cheese, in particular the Kraft Single.

3.2. *Functional evaluation of commercial dairy and plant-based cheese alternatives*

The functional properties of the commercial and plant-based cheese alternatives are presented in Table 2. The first parameter listed is hardness; the property reflects the mechanical strength of the cheese. The two dairy cheeses exhibit notably different hardness levels, with cheddar significantly harder at 74N than the processed Kraft Single at 22N. The commercial plant-based alternatives also had a range of hardness values, with two of the tested samples reaching up to 100N, which is significantly harder than both commercial dairy samples. Daiya cheese was the softest commercial plant-based sample, reaching a hardness of 56N, which was still considerably harder than a Kraft Single but not hard enough to be similar to conventional cheddar. Only Violife cheddar-style slices, which had a hardness of 89N, was statistically similar to commercial cheddar.

Table 2

Functional properties of commercial dairy cheese, commercial plant-based cheese alternatives, and novel high-protein plant-based cheese alternative. Values are mean ± standard deviation, $n \geq 3$; samples with the same lowercase letter within the same column are not statistically different, $P > 0.05$.

Sample	*Hardness (N)*	*Melt (%)*	*Oil loss (%)*	*Stretch(mm)*
Dairy				
Kraft Single	22.1 ± 2.1^{ef}	146.8 ± 6.4^{b}	0^{e}	36.3 ± 11.2^{b}
Medium Cheddar	74.9 ± 16.5^{bc}	184.5 ± 2.1^{a}	100^{a}	95.2 ± 0.01^{a}
Plant-Based				
Daiya Cheddar	56.7 ± 9.9^{d}	1.3 ± 0.5^{f}	0^{e}	8.1 ± 2.9^{c}
Violife Cheddar	89.8 ± 22.9^{ab}	13.3 ± 1.0^{ef}	0^{e}	11.9 ± 6.8^{c}
EarthIsland Cheddar	101.9 ± 9.3^{a}	5.7 ± 4.5^{f}	0^{e}	6.0 ± 1.7^{c}
Sheese Cheddar	100.3 ± 13.4^{a}	24.5 ± 5.8^{e}	0^{e}	16.7 ± 4.3^{c}
Novel Formulation	*High-Protein Plant-Based*			
Heating time: T3	16.1 ± 5.2^{f}	92.1 ± 6.9^{cd}	44.3 ± 1.4^{d}	34.8 ± 8.7^{b}
T4	23.4 ± 8.5^{ef}	102.0 ± 5.3^{c}	78.3 ± 7.2^{c}	39.2 ± 7.0^{b}
T5	37.1 ± 13.1^{e}	96.3 ± 0.5^{c}	71.7 ± 5.8^{c}	37.4 ± 5.6^{b}
T6	61.0 ± 16.9^{cd}	95.0 ± 8.0^{c}	88.7 ± 3.7^{b}	33.8 ± 5.8^{b}
T7	76.4 ± 10.6^{bc}	75.8 ± 10.5^{d}	48.2 ± 4.7^{d}	33.6 ± 5.6^{b}

The high-protein plant-based cheese alternative formulation, however, reached a range of hardness levels both statistically similar to a Kraft Single and Commercial Chedder, depending on the duration of heating mixing (Table 2). The range of hardness values can be attributed to the mixing technique. The process is similar to a typical starch pasting curve; in our system, low shear mixing allows the native starch granules to swell, creating structure and increasing viscosity (Kumar and Khatkar, 2017; Tappiban et al., 2020). The continued heating and short high shear mixing segments then induced the breakdown segment of pasting where swollen granules are disintegrated, and, in our case, the added high shear enhanced the viscoelastic and stickiness properties of waxy starch (Kumar and Khatkar, 2017; Tappiban et al., 2020). The high-protein system is then continued to be heated for different durations of time depending on the desired sample hardness. Samples taken during early time periods (Time 3, 4, and 5) have more native and swollen granules remaining, as seen by increased birefringence observed in polarized light microscopy (Supplementary Fig. 2). The intact starch granules indicate that there is less gelatinized starch available for starch-starch interaction and reorganization that occur during retrogradation(Li et al., 2015; Srichuwong et al., 2005). The limited hardness can thus be attributed to a lower concentration of gelatinized starch. The relationship of starch concentration to gel strength has been observed by many researchers, where greater starch concentration produces stronger gels (Li et al., 2015; Mandala and Palogou, 2003). It is also hypothesized that the remaining native starch granules could act as particulate fillers in conjunction with protein and interrupt starch-starch interactions. The application of starch as fillers was observed by Gravelle et al. (2017), where insufficient free water resulted in partially swollen granules distributed throughout the gel network. The starch acted as a particulate filler instead of forming gel or interacting with the surrounding network. I Samples taken after longer heating durations (Time 6 and 7) have fewer remaining native granules and increased granule disintegration. Therefore, during retrogradation, the cheese network has a greater concentration of gelatinized starch and fewer particulate fillers interrupting recrystallization where the structures can compact more, causing an increase in sample hardness.

The meltability and spread of cheese is widely recognized as a desirable attribute. The percent melt of the samples is listed in Table 2. The measurement reflects the sample spread after heating, where an increase in the area indicates superior melting. The melting values for dairy cheese are very large, reaching upwards of 184% spread for conventional cheddar and 146% for a Kraft Single. The modified Schreiber melt test for a Kraft Single can be seen in Fig. 1A, where the sample displays a large spread after heating. In cheese, the protein casein has the unique ability to soften and spread due to the weakening of the protein network, giving typical dairy cheese the well known ability to melt (Lamichhane et al., 2018; Lucey et al., 2003). As for the commercial plant-based cheese alternatives, they all displayed significantly lower meltability than commercial dairy. Daiya and EarthIsland cheddar displayed 1.3% and 5.7% melts, respectively. Violife and Scheese were only marginally better at 13.3% and 24.5% melt, respectively. The Schreiber melt test for Daiya cheese can be seen in Fig. 1B. The sample looks as though it did not change in diameter after heating which is consistent with the 1.3% melt value. The Daiya cheese was selected for comparison as it had the lowest hardness making it the cheese closest in hardness to a Kraft Single. The poor meltability of plant-based cheese alternatives has been recognized by other researchers and is a common negative attribute listed in perception studies (Falkeisen et al., 2022; Grasso et al., 2021). The lack of meltability of the plant-based samples can be attributed to the ingredients used in the formulations, specifically the

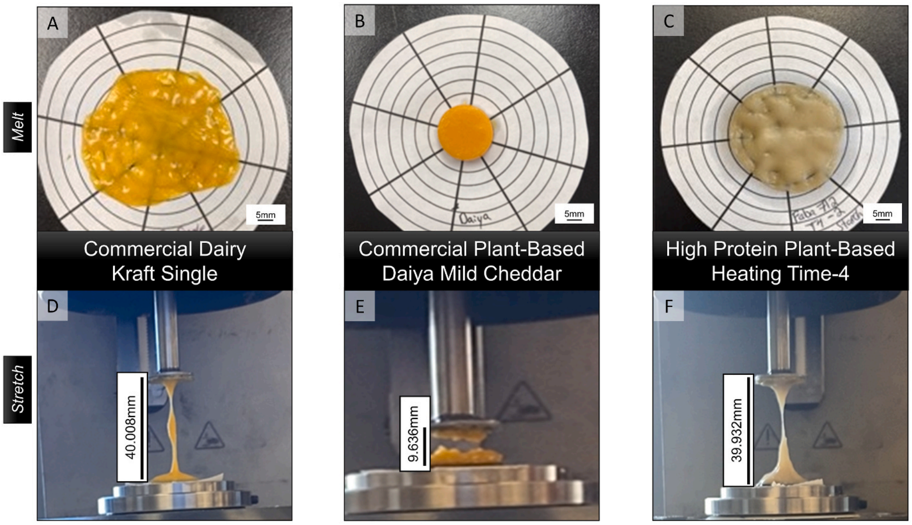

Fig. 1. Melt images and max stretch video captures. A and D) commercial processed dairy cheese. B and E) commercial plant-based cheese alternative. C and F) novel high-protein plant-based cheese alternative. Stretch videos for images D, E and F can be found in supplementary data, Movie S1, Movie S2 and Movie S3 respectively.

starches. If the starches contain amylose, they tend to have poor softening properties as amylose forms irreversible gels during retrogradation. Starches high in amylopectin, also known as waxy starches, have more reversible properties after retrogradation and demonstrate more softening, but the gels that the system forms are quite weak. As a result, many companies have opted to use modified starches to provide better structure; however, the melting attribute is still limited in all samples. While some softening occurs, too much irreversible structure remains, thus limiting the meltability.

High-protein plant-based cheese alternative was created in an effort to improve both nutrition and functional properties. The cheeses produced at all mixing time lengths (Table 2) have significantly greater melt than all commercial plant-based samples. The cheeses made at times T3 through T6 all had statistically similar melt reaching 95–102% spread. Sample T7 was the only cheese that displayed a slightly lower melt at 75% spread. However, this can be attributed to the greater sample hardness and corresponding increased reorganization during retrogradation and a decrease in moisture due to the extended heating time. The melt of the sample at Time 4 is presented in Fig. 1C. The sample was selected as it matches the hardness value of a Kraft Single (Fig. 1A), thus being a valid comparison to the product. The meltability reached 102% spread which was not as large as Kraft Single. Still, there are visual similarities, such as the presence of bubbles or thinning areas due to the spread of the cheese, indicating that in both samples, heat resulted in structure change or breakdown, which contributed to the sample spread.

The unique meltability of the high-protein plant-based cheese alternative can be attributed to the formulation and mixing method. The high-protein cheese utilizes native waxy corn starch as the sole starch component. The formulation incorporates protein before heating. This allows the protein to begin to swell before the starch component. As the system is slowly heated and mixed, the starch begins to swell and gelatinize, creating a viscous system for the protein to actively fill. The continuous starch phase also creates a stable network for the fat to remain dispersed. The mixing method also includes short segments of high shear. During the shearing phase, it was visually observed that the cheese decreased in viscosity and increased in stickiness. We suspect that the shear has caused granule breakdown and also possible fragmentation. Moreover, the mixing process does not exceed temperatures above 80 °C, which restricts protein denaturation and network formation. This is supported by differential scanning calorimetry completed on the fava protein (Supplementary Fig. 3) where no endothermic events occur through heating to 110 °C. Thus, the protein remains a particulate filler. The viscous system is then left to set under refrigeration, where the formulation becomes a solid gel. The resulting high-protein plant-based cheese alternative possesses a unique structure with dispersed protein and fat, ultimately aiding in the significant melt properties.

Oil loss is the next parameter listed in Table 2 and reflects the amount of oil released from the cheeses during melting. It's common for natural cheeses like cheddar to lose oil during melting. This is because the saturated milk fat turns from a solid to a liquid when heated and is released as the casein network weakens. Additionally, the product contains no emulsifiers or additional starches such as processed cheese. As listed in Table 2, mild cheddar displayed 100% oil loss, and processed Kraft Single had 0%. No oil loss was also observed for all of the commercial plant-based samples. The plant-based cheese alternatives all contain a variety of starches, many of which are modified and contribute to the oil binding of the products. The novel formulation, however, did display oil loss at all time points. The oil loss at Time 4 can be seen in Fig. 1C, where the Schreiber melt paper saturation is observed around the melted cheese. The oil loss of the high-protein cheese can be attributed to the lack of modified starches that would typically aid in emulsification. In return, the novel cheese product is clean label and displays more typical oil loss to natural dairy cheese.

The property of dairy cheese to flow and stretch is a widely recognized and valued characteristic. The stretch relies on heating to increase the hydrophobic interaction between casein molecules resulting in contraction of the protein network(Correia Gonçalves and Cardarelli, 2021; Lamichhane et al., 2018). When mechanical energy is applied the casein molecules align creating a fibrous protein network that promotes elongation and stretch (Correia Gonçalves and Cardarelli, 2021; Lamichhane et al., 2018). An extensional rheology method was developed that enabled constant heating and constant speed of elongation, providing much more accurate results than the commonly used fork test. Cheese samples are heated and held at 80 °C to reach their most viscous state, where the sample is then extended, and the length to which it can stretch is measured. As listed in, Table 2 commercial dairy, mild cheddar cheese displayed the greatest stretch, extending the maximum measurable distance of 95 mm. The Kraft Single stretched an average of 36 mm, and a snapshot of the max stretch can be observed in Fig. 1D. The cheese pull kinetics can be observed in Supplementary Movie S1, where the Kraft Single displays good connectivity and a fluid stretch. On the other hand, the commercial plant-based cheese alternatives had very minimal stretch (Table 2), with only one sample, Sheese, reaching a maximum stretch of 16.7 mm. While this value is approaching that of a Kraft Single, it is important to consider the other functional properties lacking (i.e., spread, hardness). Fig. 1E displays the cheese pull of commercial plant-based Daiya cheese and demonstrates the poor stretch properties. The full stretch can be observed in Supplementary Movie S2, where little to no sample connectivity is seen. The poor stretch coincides with the poor melt properties indicating that the sample may be overstructured, thus limiting the amount of softening that can occur. The novel high-protein cheese, on the other hand, had significantly greater stretch than all the commercial plant-based samples. All samples T3-T7 had statistically similar stretch values indicating that heated mixing time did not impact the stretch of the products. The high-protein cheeses had statistically similar stretch values to commercial dairy Kraft Single cheese. The stretch of sample T4 can be observed in Fig. 1F and supplementary Movie S3, where the sample shows good connectivity and fluid stretch. The significant stretch of the product can be attributed to the viscoelastic and reversible gelatinization of waxy maize, enabling the cheese to soften and flow. Additionally, we hypothesize that the distributed solid fat globules and plant protein create strategic junction points that, when heated, cause network collapse and allow better connectivity of the starch phase, thus enhancing the stretch of the network.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.crfs.2023.100632

3.3. Rheological evaluation of commercial dairy cheese and plant-based cheese alternatives

The rheological melting profiles for commercial dairy cheese, commercial plant-based cheese alternatives, and novel high-protein plant-based cheese alternative are displayed in Fig. 2. Dairy cheeses (Fig. 2A) and commercial plant-based cheese alternatives (Fig. 2B) exhibit vastly different melting profiles. Dairy cheeses demonstrate more continuous melt with no discriminative plateau in storage modulus (G’) or loss modulus (G”) over the heating period. Both the Kraft Single and conventional cheddar start with more solid behaviour G’>G” at lower temperatures, then begin to soften due to the initial melting of milk fat. Further softening is observed where the samples reach crossover points G’ = G” between 68 and 70 °C indicating the cheese has transformed from a solid to a viscous state. As heating continues, the samples become more fluid G”>G’ further demonstrating the melting properties associated with dairy cheese. The significant structure change during heating is also reflected in Fig. 2D. Tan δ =(G”/G’) indicates the ratio of liquid to solid behaviour and relates to the degree of melt that has occurred. As the tan δ value approaches or surpasses the value of one, the sample becomes increasingly viscous and demonstrates better melt. The dairy cheeses begin with low tan δ values, as noted in Table 3, indicating more solid behaviour. However, at ∼45 °C for a Kraft Single and ∼52 °C for cheddar, the tan δ values exponentially increase. The Y-axis in Fig. 2D has been constrained in order to better view the plant-based melting profiles. However, the Kraft Single and mild cheddar reach final tan δ values of 1.35 and 2.90 (Table 3), respectively. The values further support the superior melting and viscous state that conventional dairy cheese can reach. The melting profiles for commercial plant-based cheese alternatives (Fig. 2B) exhibit more segmented melting or, rather, softening as the samples maintain greater solid structure G’>G” throughout the entire temperature sweep. In the initial heating segment, the samples exhibit a slight decrease in G’ to 25 °C, where the G’ then decreases more rapidly until 40 °C, consistent with the melting of coconut oil. The G’ then plateaus until around 60 °C, where again the G’ then decreases more rapidly until the final temperature point of 80 °C. The second decrease in G’ correlates to the gelatinization temperature of starch, where the reversible gelatinization of waxy starch exhibits sample softening. However, the degree of sample melt/softening is minimal. As seen in Fig. 2D and further supported in Table 3, the tan δ values for the commercial plant-based cheese alternatives are very low and show very little increase over the duration of heating. Based on the

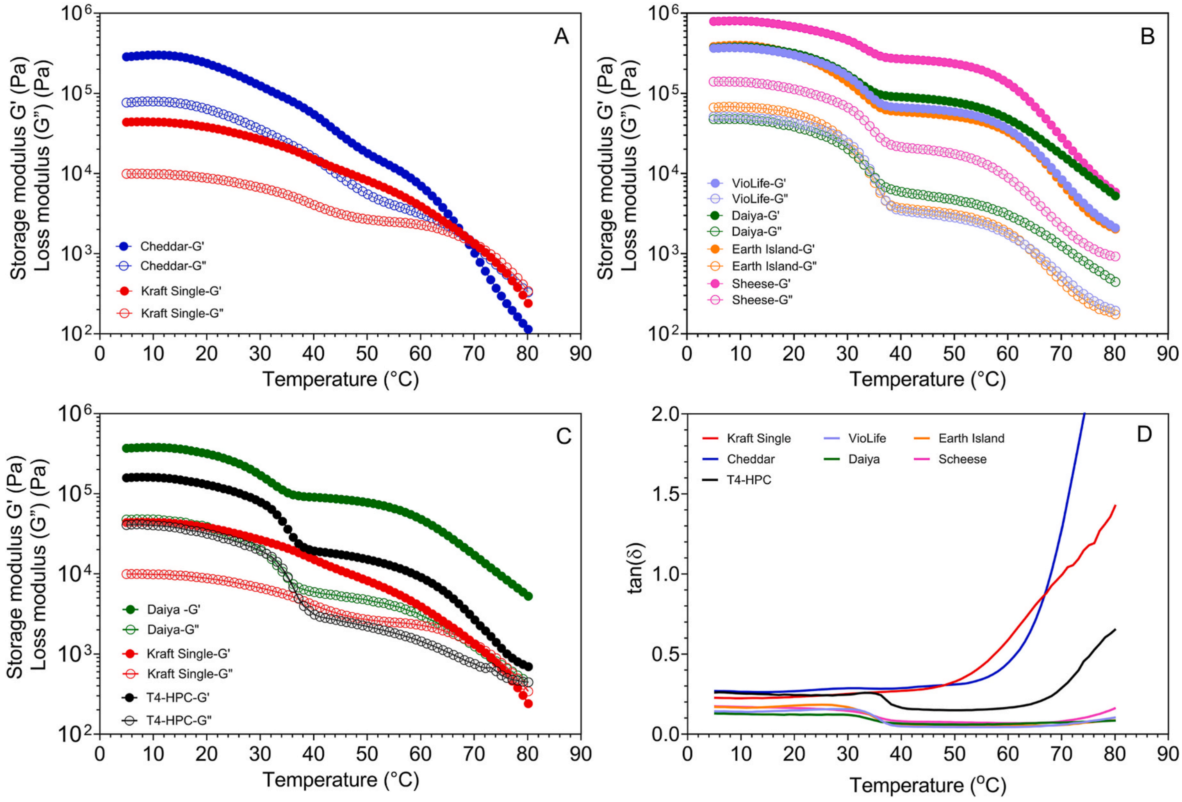

Fig. 2. *Rheological melting profiles, Storage (G’) and loss modulus (G”) over increasing temperature from 5-80°C for A) Cheddar and Kraft Single, B) Violife, Daiya, Earth Island and Sheese, C) Kraft Single, Daiya, and High-protein plant-based cheese alternative heating time -T4. D)* Tanδ= (G”/G’) *of all commercial samples and High-protein plant-based cheese alternative heating time -T4, over increasing temperature from 5-80°C. Values are the mean n = 4.*

Table 3
Rheological melting parameters of commercial dairy and plant-based cheese alternatives at different temperature points, Storage modulus (G'), Loss modulus (G''), Tan δ (G''/G'). Values are the mean ± standard deviation, n = 4. Values with the same lowercase letter in the same column are not significantly different $p > 0.05$.

Sample		Tan δ	
Temperature		40 °C	80 °C
Dairy			
	Kraft Single	0.27 ± 0.01^{a}	1.35 ± 0.24^{b}
	Medium Cheddar	0.29 ± 0.01^{a}	2.90 ± 0.11^{a}
Plant-Based			
	Daiya Cheddar	0.06 ± 0.01^{d}	0.08 ± 0.01^{g}
	Violife Cheddar	0.05 ± 0.01^{d}	0.10 ± 0.03^{g}
	EarthIsland Cheddar	0.06 ± 0.01^{d}	0.09 ± 0.01^{g}
	Sheese Cheddar	0.08 ± 0.01^{d}	0.16 ± 0.02^{g}
Novel Formulation	*High-Protein Plant-Based*		
Heating time:	T3	0.19 ± 0.03^{b}	0.61 ± 0.06^{cd}
	T4	0.16 ± 0.01^{b}	0.66 ± 0.07^{c}
	T5	0.14 ± 0.01^{c}	0.62 ± 0.08^{ce}
	T6	0.14 ± 0.01^{c}	0.47 ± 0.14^{def}
	T7	0.13 ± 0.01^{c}	0.51 ± 0.10^{cf}

low tan δ values, the tested plant-based samples have very little to no viscous properties. In addition, the rheological melting profiles and poor sample melt and stretch observed in section 3.2 confirm that these samples are quite dissimilar to commercial dairy cheese.

The melting profile for the novel high-protein plant-based cheese alternative, sample T4, can be observed in Fig. 2C. The melting plot includes the melting profiles for commercial dairy cheese, Kraft Single and commercial plant-based Daiya cheese. The samples were selected for comparison to complement Fig. 1 and due to the previously determined similarities in hardness, melt and stretch of the samples. The novel high-protein cheese exhibited similar segmented melting as the commercial plant-based samples. The G' initially decreased due to coconut oil melting, followed by a second decrease in G' related to starch softening and gelatinization. However, the distinct difference was that the slope of G'' is lower than the G' therefore they are approaching each other. We attribute this change to protein being incorporated into the cheese, resulting in structural changes that enhance the viscous state. The addition of protein could be acting as a passive filler which we hypothesize impacted the initial retrogradation and gel structure, creating points of network weakening and allowing the sample to reach a more viscous state when heated. The tan δ curve, as seen in Fig. 2D, and values presented in Table 3 also demonstrate the viscous property, as the tan δ increases with increasing temperature. While the tan δ values at 80 °C are not as large as conventional dairy cheese, only reaching values of 0.50–0.66, the values are significantly greater than all commercial plant-based samples, which had one sample only reach a maximum tan δ value of 0.16. The increase in viscous modulus of the high-protein plant-based cheese alternative further supports the observed superior melting and stretch properties.

4. Conclusion

The existing gap between commercial dairy and commercial plant-based cheese alternatives is evident. The study demonstrated that current plant-based products lack nutritionally due to the low protein content as well as functionally having poor; melt, stretch and mechanical sensory attributes. The current plant-based alternatives have no aligning characteristics with dairy cheese. The successful development of the novel high-protein cheese signifies a breakthrough in the food sector. The high-protein product was able to match the protein level of a commercial Kraft Single, creating a nutritionally competitive product. The functional properties of the novel cheese were also able to outperform all commercial plant-based options. Incorporating protein, paired with heated mixing, and using the existing characteristics of the ingredients, enabled the novel formulation to reach a range of hardness levels that could accurately match that of commercial cheddar or processed cheese. The novel formulation also had superior melt to all commercial plant-based samples but not as extreme as dairy cheese and is an area set for extended research. The stretch of the sample, however, matched that of a Kraft Single, again demonstrating commercial parity. Ultimately continued research and innovation in this field hold the key to creating even more convincing and appealing plant-based cheese alternatives, contributing to a healthier, more environmentally friendly, and inclusive food landscape.

CRediT authorship contribution statement

S. Dobson: Conceptualization, Methodology, Formal analysis, Writing – original draft, Visualization. **A.G. Marangoni:** Conceptualization, Methodology, Validation, Supervision, Writing – review & editing, Funding acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2023.100632.

References

Caric, M., Gantar, M., Kalab, M., 1985. Effects of emulsifying agents on the microstructure and other characteristics of process cheese - a review. Food Microstruct. Struct. 4 (2), 297–312.

Correia Gonçalves, M., Cardarelli, H.R., 2021. Mozzarella cheese stretching: a review. Food Technol. Biotechnol. 59 (1), 82–91. https://doi.org/10.17113/ftb.59.01.21.6707.

Dobson, S., Laredo, T., Marangoni, A.G., 2022. Particle filled protein-starch composites as the basis for plant-based meat analogues. Curr. Res. Food Sci. 5 (May), 892–903. https://doi.org/10.1016/j.crfs.2022.05.006.

Falkeisen, A., Gorman, M., Knowles, S., Barker, S., Moss, R., Mcsweeney, M.B., 2022. Consumer perception and emotional responses to plant-based cheeses. Food Res. Int. 158, 111513 https://doi.org/10.1016/j.foodres.2022.111513.

Fresán, U., Rippin, H., 2021. Nutritional quality of plant-based cheese available in Spanish supermarkets: how do they compare to dairy cheese? Nutrients 13 (9), 3291. https://doi.org/10.3390/nu13093291.

GFI, 2021. Retail Sales Data: Plant-Based Meat, Eggs, Dairy. GFI.

Grasso, N., Roos, Y.H., Crowley, S.V., Arendt, E.K., O'Mahony, J.A., 2021. Composition and physicochemical properties of commercial plant-based block-style products as alternatives to cheese. Future Foods 4, 100048. https://doi.org/10.1016/j.fufo.2021.100048.

Gravelle, A.J., Barbut, S., Marangoni, A.G., 2017. Food-grade filler particles as an alternative method to modify the texture and stability of myofibrillar gels. Sci. Rep. 7 (1), 11544 https://doi.org/10.1038/s41598-017-11711-1.

Grossmann, L., McClements, D.J., 2021. The science of plant-based foods: approaches to create nutritious and sustainable plant-based cheese analogs. Trends Food Sci. Technol. 118, 207–229. https://doi.org/10.1016/j.tifs.2021.10.004.

Kumar, R., Khatkar, B.S., 2017. Thermal, pasting and morphological properties of starch granules of wheat (Triticum aestivum L.) varieties. J. Food Sci. Technol. 54 (8), 2403–2410. https://doi.org/10.1007/s13197-017-2681-x.

Lamichhane, P., Kelly, A.L., Sheehan, J.J., 2018. Symposium review: structure-function relationships in cheese. J. Dairy Sci. 101 (3), 2692–2709. https://doi.org/10.3168/jds.2017-13386.

Li, Z., Liu, W., Gu, Z., Li, C., Hong, Y., Cheng, L., 2015. The effect of starch concentration on the gelatinization and liquefaction of corn starch. Food Hydrocolloids 48, 189–196. https://doi.org/10.1016/j.foodhyd.2015.02.030.

Lucey, J.A., Johnson, M.E., Horne, D.S., 2003. Invited review: perspectives on the basis of the rheology and texture properties of cheese. J. Dairy Sci. 86 (9), 2725–2743. https://doi.org/10.3168/jds.S0022-0302(03)73869-7.

Mandala, I.G., Palogou, E.D., 2003. Effect of preparation conditions and starch/xanthan concentration on gelation process of potato starch systems. Int. J. Food Prop. 6 (2), 311–328. https://doi.org/10.1081/JFP-120017818.

Mattice, K.D., Marangoni, A.G., 2020a. Evaluating the use of zein in structuring plant-based products. Curr. Res. Food Sci. 3, 59–66. https://doi.org/10.1016/j.crfs.2020.03.004.

Mattice, K.D., Marangoni, A.G., 2020b. Physical properties of plant-based cheese products produced with zein. Food Hydrocolloids 105 (January), 105746. https://doi.org/10.1016/j.foodhyd.2020.105746.

Ritchie, H., Rosado, P., Roser, M., 2022. *Environmental Impacts of Food Production*. Our World in Data.

Saraco, M., 2021. Functionality of the Ingredients Used in Commercial Dairy-free Imitation Cheese and Analysis of Cost-Related, Food Safety and Legal Implications (Issue October 2019).

Short, E.C., Kinchla, A.J., Nolden, A.A., 2021. Plant-based cheeses: a systematic review of sensory evaluation studies and strategies to increase consumer acceptance. Foods 10 (4), 1–12. https://doi.org/10.3390/foods10040725.

Singhal, A., Karaca, A.C., Tyler, R., Nickerson, M., 2016. Pulse proteins: from processing to structure-function relationships. Grain Legumes. https://doi.org/10.5772/64020.

Srichuwong, S., Sunarti, T.C., Mishima, T., Isono, N., Hisamatsu, M., 2005. Starches from different botanical sources II: contribution of starch structure to swelling and pasting properties. Carbohydr. Polym. 62 (1), 25–34. https://doi.org/10.1016/j.carbpol.2005.07.003.

Tappiban, P., Ying, Y., Pang, Y., Sraphet, S., Srisawad, N., Smith, D.R., Wu, P., Triwitayakorn, K., Bao, J., 2020. Gelatinization, pasting and retrogradation properties and molecular fine structure of starches from seven cassava cultivars. Int. J. Biol. Macromol. 150, 831–838. https://doi.org/10.1016/j.ijbiomac.2020.02.119.