Innovative Food Science and Emerging Technologies 96 (2024) 103750

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

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

# Optimization of pulsed electric field processing to reduce the viscosity of micellar casein concentrate

Sonali Raghunath [a], Tonya Schoenfuss [b], Kumar Mallikarjunan [c,*]

[a] Graduate Student, Department of Food Science and Nutrition, University of Minnesota - Twin Cities, Saint Paul, MN 55108, USA
[b] Associate Professor, Department of Food Science and Nutrition, University of Minnesota - Twin Cities, Saint Paul, MN 55108, United States of America
[c] Professor of food engineering, Department of Food Science and Nutrition, University of Minnesota - Twin Cities, Saint Paul, MN 55108, United States of America

ARTICLE INFO

*Keywords:*
Pulsed electric field
Micellar casein concentrate
Viscosity
Response surface methodology
Optimization

ABSTRACT

Due to its high casein content, micellar casein concentrate (MCC) is a stable protein currently used for various product applications. Our objective was to reduce the viscosity of MCC using a pulsed electric field (PEF) processing which is one of the non-thermal technologies researched in the market. In this study, the effect of processing conditions for PEF treatment, such as temperature (15–45 °C), electric field strength (EFS) (4–20 kV/cm), and frequency on the viscosity (30–300 Hz) of MCC was investigated and optimized using response surface methodology (RSM). The analysis resulted in a quadratic prediction model with $R^2 = 0.91$. The optimized conditions were 35 °C, EFS at 4 kV/cm and frequency at 63 Hz. The optimized consistency coefficient was predicted to be 1440.57 Pa $s^n$ which was 46% less than control at 30 °C. Temperature and EFS were found to be the most critical parameters that affect the functionality.

*Industrial relevance:* This study provides the optimized process conditions for reducing the viscosity of MCC using PEF, which would benefit the application of MCC in various end-product applications. The results indicate the relevance of using PEF as a treatment through an inline process during the manufacturing of MCC which will in turn allow the dairy industry to fine tune the ingredients and lead to the production of novel ingredients with enhanced functionality.

## 1. Introduction

Most American consumers seek increased protein but of good quality in their everyday diet (Dunn, Barbano, & Drake, 2021). In addition to protein-rich ingredients, customers also seek clean ingredients and minimally processed foods. Dairy proteins being the most desirable has led to an increase in milk proteins, a growing segment in the protein market, and is expected to increase in the future (Hammam, Martínez-Monteagudo, & Metzger, 2021; Lagrange, Whitsett, & Burris, 2015). Various milk proteins are available in the market depending on their end product usage and the desired functional attributes. The most commonly used dairy protein ingredients are milk protein concentrates, isolates, caseinates, and micellar casein concentrates (MCC) (Dunn et al., 2021). Micellar casein has been gaining attention lately due to its unique features like high casein content (Beliciu, Sauer, & Moraru, 2012; Pellegrino, Masotti, Cattaneo, Hogenboom, & De Noni, 2013), cold set gels, and heat stability. They are predominantly used in beverages that involve sterilization without affecting the native quality of the protein (Beliciu et al., 2012).

MCC are high-protein ingredients, generally manufactured in stages with microfiltration and diafiltration processes (Hammam & Metzger, 2023) and have been made possible through membrane processing. Various methods, such as acidification, rennet utilization, and co-precipitation, are utilized in the production of other casein or protein sources (Beliciu et al., 2012), resulting in distinctive characteristics and protein-related challenges (Beliciu et al., 2012; Grossbier, 2016; Hurt, Zulewska, Newbold, & Barbano, 2010; Nelson & Barbano, 2005). MCC, in particular are obtained through microfiltration, mainly consisting of CN (casein) in micellar form, lactose, minerals, and serum proteins (Beckman, Zulewska, Newbold, & Barbano, 2010). Skim milk is sent through a membrane where casein will be on the retentate, and serum proteins will be the permeate based on size. The retentate is termed MCC and consists mainly of casein (Hammam & Metzger, 2023). The serum proteins will be removed from the milk up to 95%. The final composition

* Corresponding author at: University of Minnesota, College of Food, Agriculture and Natural Resource Sciences, Department of Food Science and Nutrition, Saint Paul, MN 55108, USA.
*E-mail address:* kumarpm@umn.edu (K. Mallikarjunan).

https://doi.org/10.1016/j.ifset.2024.103750
Received 20 May 2024; Received in revised form 7 July 2024; Accepted 8 July 2024
Available online 11 July 2024

of MCC is dependent on the end-product use.

Due to the inherent properties of casein proteins they are used as ingredients (Hammam et al., 2021) in confectionaries, meat, and cheese (Hammam et al., 2021; Hammam, Kapoor, & Metzger, 2023; Hammam & Metzger, 2023); meal replacers, nutritional products, whipped toppings, coffee whiteners, and healthy add-ons (Beckman et al., 2010). MCC's are known to form thermally reversible gels (Dunn et al., 2021) and are generally tricky to rehydrate. Due to this phenomenon, MCC exhibits challenging functional properties as affected by process conditions such as time and temperature (Dunn et al., 2021; Nasser et al., 2017a, Nasser, Moreau, Jeantet, Hedoux & Delaplace, 2017b). The viscosity characteristic of MCC increased with increased protein content and higher casein proteins (Dunn et al., 2021; Misawa, Barbano, & Drake, 2016). Casein proteins are known for their reduced solubility due to non-polar residues; however, this issue is mitigated by the presence of carbohydrates in kappa casein and a low sulfhydryl content (Huppertz, 2013; McMahon & Oommen, 2013; McSweeney & Fox, 2013; O' Mahony & Fox, 2013). MCC is well-known for its heat stability (Beliciu et al., 2012; Hammam et al., 2023) and has a rapid onset of cold gelation and thickening with time. These functionality variations are not entirely explained in the literature. However, there are various theories about the phenomena (Dunn et al., 2021).

MCC faces challenges during processing due to its highly viscous nature and susceptibility to gelling. This means that drying MCC is costly as the spray dryer uses much more energy to remove water from highly viscous fluids than evaporators for water removal. The primary issues faced during the product formulations of MCC involve viscosity and the formation of low-concentration gels, and there has been constant exploration around improving the viscosity of MCC for ingredient applications. For reducing viscosity of MCC to be used as ingredient in product formulations, many methods, including chemical, physical, and enzymatic modification, have been explored, but with drawbacks. Due to this, there is an increasing value and demand for alternative process technologies such as ultrasound (Schulnies, Höhme, & Kleinschmidt, 2023) and pulsed electric fields (PEF) (Taha et al., 2023).

PEF treatment utilizes electric fields to alter functional properties such as viscosity, particle size, and gelation (Syed, Ishaq, Rahman, Aslam, & Shukat, 2017). PEF improves the functional properties of proteins through structural modifications, minimizing nutrition loss, reducing the microbial load, and improving ingredients' quality. Specific effects of PEF on proteins would also depend on processing parameters (Taha et al., 2022). Previous studies have shown mixed results on dairy protein ingredients. Protein studies on PEF have caused no significant changes in the covalent bond group, surface hydrophobicity, and protein unfolding behavior, and the droplet size remained the same in the case of Whey Protein Isolate (Sui, Roginski, Williams, Versteeg, & Wan, 2011). In another study, PEF treatment improved surface hydrophobicity and caused secondary structure alteration (Rodrigues, Avelar, Vicente, Petersen, & Pereira, 2020) for whey proteins combined with ohmic heating. It has also been reported that treatments with PEF on milk have reduced viscosity (Xiang, Simpson, Ngadi and Simpson, 2011) with enhanced coagulation properties of proteins due to a decrease in the size of the casein micelle with higher electric field strength (EFS) (Floury et al., 2006; Hemar et al., 2011). They have also been shown to reduce viscosity and smaller aggregates for whey proteins (Rodrigues et al., 2020). It was also reported to protect the integrity of milk proteins due to reduced unfolding (Sharma, Oey, & Everett, 2016). The modifications of charges in casein micelle and ionic interaction between casein in milk protein were also observed with PEF processing. (Hemar et al., 2011). However, with BSA and applying PEF, the tertiary structure changed with EFS, leading to structural changes in hydrogen bonds (Bekard & Dunstan, 2013). All these studies have indicated various levels of PEF treatment used, different systems at batch or lab scale, and more research is needed into the practical application and translatability of the industrial scale approach. Additionally, while the effect of PEF on various proteins was studied, more research needs to be done on optimizing process parameters for PEF to have desirable functional properties for dairy proteins, such as reducing the viscosity of MCC.

As PEF has been demonstrated to alter protein structure and reduce viscosity, this study aimed to investigate the effect of processing parameters of PEF processing on the rheological properties of MCC and optimize the parameters to obtain a maximum reduction in the viscosity of MCC. This could allow for MCC to be used in end-product applications and potentially modify its functional properties.

## 2. Materials and methods

### 2.1. Chemicals and reagents

The Imperial™ protein stain was bought from Thermo Scientific in Waltham, MA. Calcium Chloride Anhydrous ($CaCl_2$) was sourced from Fisher Scientific in Waltham, MA. β-mercaptoethanol and Sodium Dodecyl Sulfate were utilized from Sigma Life Sciences, based in St. Louis, MO. Two times of Laemmli sample buffer, the Precision Plus protein standard, TGX Precast Gels (4–20%), and 10 times of Tris/Glycine/SDS Buffer were acquired from Bio-Rad Laboratories in Hercules, CA.

### 2.2. PEF treatment

Commercial liquid MCC with 21% TS was obtained post-filtration from local dairy supplier. Based on the certificate of Analysis provided by the supplier the proximate composition of MCC was Protein (86.1%), fat (1.5%), ash (7.1%), and moisture (4.3%).Pulsed electric treatment was applied based on the experimental design created using JMP Pro 17 ® (JMP, Version 17, JMP Statistical Discovery LLC, Cary, NC) and Design of Experiments software (Stat-Ease360, Version 14, Stat-Ease Inc., Minneapolis, MN). To assess PEF's impact on MCC, samples were exposed to various process conditions: temperature (15–45 °C), EFS (4–20 kV/cm), and frequency (30–300 Hz) as per prior research (Raghunath, Mallikarjunan, & Schoenfuss, 2021). Constants included a flow rate of 106.7 L/h, pulse width of 20 μs, and electrode gap of 10 mm. Inlet and outlet temperatures were monitored to understand system-generated heat. For each treatment, 11.34 kgs (25 lbs.) of the sample were processed through a heat exchanger (Direct/Indirect UHT/HTST Series, Microthermics®, Raleigh, NC) followed by PEF treatment. An industrial-scale continuous PEF system was used (ELEA El-Crack®, HVP 5, German Institute of Food Technologists, Quakenbrück, Germany) to apply the conditions per the experimental design. Post-PEF, samples underwent spray drying (Compact spray dryer type 1, APV Anhydro, Tonawanda, NY) with operating parameters: inlet temperature 170 °C, outlet temperature 96 °C, and atomizer speed 2500 rpm. The PEF system was cleaned after each run to prevent cross-contamination. Control samples at 15, 30, and 45 °C underwent heat treatment using the heat exchanger without PEF treatment. All dry PEF treated/control MCC samples were stored at 25 °C for analysis.

### 2.3. Apparent viscosity

Viscosity testing was carried out with Anton Paar Rheo Compass™ MCR 92 S/N 82312459 (Anton Paar GmhH, 8054 Graz, Austria) with a jacketed bob and cup fixture. A concentric cylinder (part number 6670) with an active length of 60 mm and a bob diameter of 42 mm was used for testing. For this, 12% protein samples were prepared from dry PEF treated MCC samples and mixed thoroughly and allowed to stand at room temperature for 15 min to equilibrate the condition (Dunn et al., 2021). Each sample was tested in triplicates and was analyzed for shear stress, shear rate, viscosity, and torque. The apparent viscosity for each shear rate was log-transformed, and calculations were performed.

### 2.4. Molecular weight profile

Protein profiling of MCC samples was conducted following the established method previously detailed by Walter, Greenberg, Sriramarao, and Ismail (2016). The 10 μL of Precision Plus Protein Standard (MW 10–250 kDa) was loaded onto 4–20% precast Tris Glycine SDS gels. Then, 100 μL of 1% samples diluted with 100 μL of distilled water were utilized for the samples. For non-reducing gels, 100 μL of the diluted sample was mixed with 100 μL of 2× Laemmli buffer. On the other hand, in the case of reducing gels, a combination of 100 μL of diluted samples, 95 μL of Laemmli buffer, and 5 μL of β-mercaptoethanol was used. The treated samples were kept at 75 °C for 15 min and further cooled to 25 °C. A volume of 5 μL from these samples was loaded onto the gels. Electrophoresis of the gel was performed at 200 V, followed by staining using Imperial™ Protein Stain. De-staining was conducted using distilled water for 48 h. The Molecular Imager Gel Doc XR system (Bio-Rad Laboratories, Hercules, CA) was used to scan and analyze gels.

### 2.5. Flowability

The revolution powder analyzer (Mercury Scientific Inc., Newtown, CT, USA) assessed the flowability of the protein powders. For this test, 20 g sample was carefully weighed and transferred into a 100 cc drum for testing. The testing parameters were rotation rate of 0.3 RPM, an imaging rate set at ten frames per second (fps), a preparation time of 200 s, and the test concluded automatically after 128 avalanches were recorded. The analysis centered on evaluating the energy of these avalanches, providing valuable insights into the flowability characteristics of the samples.

### 2.6. Particle size analysis

Utilizing the Horiba Laser Scattering Particle Size Distribution Analyzer (LA - 960), specifically the Horiba Scientific Partica model (Horiba Scientific, Piscataway, NJ), particle size distribution analysis of the protein powders was conducted. For this, 0.5 g of the sample was added to the analyzer for each analysis. Before analysis, the refractive index was calibrated to 1.39, a value sourced from the work of Crowley, Gazi, Kelly, Huppertz, and O'Mahony (2014) for MCC. A triplicate analysis approach established the resultant mean particle size, enabling a comprehensive data comparison.

### 2.7. Response surface methodology (RSM)

The consistency coefficient was calculated from shear stress and apparent viscosity. The consistency coefficient was considered as an individual response or dependent variable and used to predict viscosity using RSM. The optimum conditions of PEF processing parameters like temperature, electric field strength, and frequency were considered independent variables for reducing viscosity and were predicted with maximum desirability using Design of Experiments software (Stat-Ease360, Version 14, Stat-Ease Inc., Minneapolis, MN). The temperature as $X_1$, EFS as $X_2$, and frequency $X_3$ were analyzed at three different levels to investigate and optimize the reduced viscosity of MCC. Based on the previous study, the operating parameters were selected for modeling the data: temperature: 15 to 45 °C, electric field strength: 4 to 20 kV/cm, and frequency: 30 to 300 Hz at three different levels. Table 1 represents the data matrix with designs with all the natural and coded variables. The parameters' highest and lowest values are coded as +1 and − 1, with the mid value of the sample coded as 0. A central composite face-centered design system with 18 experiments to explore the effects of PEF parameters on viscosity (i.e., consistency coefficient) was investigated (Table 2).

The results that were obtained were fitted into a polynomial equation. The coefficients of responses were analyzed with analysis of variance (ANOVA), 95% confidence interval. The interaction profiles and actual vs predicted plots were analyzed using JMP Pro 17 ® (JMP, Version 17, JMP Statistical Discovery LLC, Cary, NC).

**Table 1**
Natural and coded levels of independent and dependent variables PEF processing of MCC.

| Independent variables | Coded levels | | |
|---|---|---|---|
| | −1 | 0 | +1 |
| | Natural levels | | |
| Temperature (°C) | 15 | 30 | 45 |
| EFS(kV/cm) | 4 | 12 | 20 |
| Frequency (Hz) | 30 | 165 | 300 |

**Table 2**
Face-centered central composite design for industrial scale PEF treatment of MCC.

| Runs | Temperature (°C) | EFS (kV/cm) | Frequency (Hz) |
|---|---|---|---|
| 1 | 45 | 12 | 165 |
| 2 | 45 | 20 | 30 |
| 3 | 15 | 12 | 165 |
| 4 | 30 | 4 | 165 |
| 5 | 30 | 12 | 165 (Center) |
| 6 | 45 | 4 | 30 |
| 7 | 15 | 20 | 30 |
| 8 | 15 | 4 | 30 |
| 9 | 45 | 4 | 300 |
| 10 | 30 | 20 | 165 |
| 11 | 15 | 4 | 300 |
| 12 | 15 | 20 | 300 |
| 13 | 30 | 12 | 300 |
| 14 | 45 | 20 | 300 |
| 15 | 30 | 12 | 165 (Center) |
| 16 | 30 | 12 | 30 |
| 17 | 30 | 12 | 165 (Center) |
| 18 | 30 | 12 | 165 (Center) |
| 19 | 15 | 0 | 0 (Control) |
| 20 | 30 | 0 | 0 (Control) |
| 21 | 45 | 0 | 0 (Control) |

The obtained experimental results were fitted into the following quadratic equation as

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{23} BC + \beta_{13} AC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 \quad (1)$$

In the equation, Y- dependent variable.

*A, B, and C*– Independent variables - Temperature, electric field strength, frequency.

$\beta_0$ - constant-coefficient.

$\beta_1$, $\beta_2$, and $\beta_3$ - linear regression coefficients.

$\beta_{11}$, $\beta_{22}$, and $\beta_{33}$ - regression coefficients that are squared.

$\beta_{12}$, $\beta_{13}$, and $\beta_{23}$ - interaction coefficients of the independent variables.

## 3. Results and discussion

### 3.1. Process analysis

#### 3.1.1. Retention time

The total retention or residence time for the MCC samples per run in the PEF chamber was calculated to be 26 ms using the formula.

$$\tau = L/v = \frac{L \times A}{Q} \quad (2)$$

where,

$\tau$ – Retention time (ms).

v – Velocity (m/s).

A – Cross-sectional area ($mm^2$).

Q = V/t – Volumetric flow rate (L/s).

L – Length (mm).

The following values, diameter 10 mm, flow rate 106.7 L/h, and length 10 mm, were used to calculate the retention time based on the experimental setup.

### 3.1.2. Temperature changes in MCC due to PEF treatment

The inlet and outlet temperatures for all the MCC samples were monitored throughout the treatment process to understand the thermal energy footprint during the PEF treatment. The inlet and outlet temperatures for MCC are shown in Table 3. Based on the results, there was a minimum rise of 1.11 °C on average and a maximum of 12.50 °C in temperature after the PEF treatment. This is consistent with previous studies with MPC, where temperature rise was influenced by energy input for higher EFS and frequency (Raghunath, Mallikarjunan, & Schoenfuss, 2024). The study on casein micelles by Taha et al. (2023) also observed a slight increase in temperature after the PEF treatment. The temperature increase could affect protein denaturation; however, the quantity of whey protein in MCC is much lower compared to MPC. Since whey denaturation occurs at 65 °C (Li, Zhao, Li, & Yu, 2021) and casein protein undergoes changes at more significant than 100 °C (Farooq, 2019), the denaturation effect due to PEF treatment with MCC can be considered negligible as the maximum temperature after treatment was ≤47 °C. The casein micelles also tend to offer more resistance to heat treatment than whey; thus, the effect on MCC can be considered minimal (Taha et al., 2023). To understand that the protein quality is still maintained after PEF treatment, SDS PAGE profiling was performed. Therefore, the observed temperature rise for MCC after the PEF treatment could be seen as a minimal effect on the casein stability as a protein.

## 3.2. Apparent viscosity analysis

The effect of PEF processing on the viscosity of MCC was analyzed using a face-centered central composite design, and Table 4 represents the results obtained. In theory, many models describe the relationship between viscosity and shear rate, including the power law, Bingham, and Herschel Bulkley models. Different equations have been used to understand the nonlinear flow behavior of micellar casein concentrate; however, due to its simplicity and applicability, the power law model has been used for this study (Kieferle, Hiller, Kulozik, & Germann, 2019). Eq. (3) was selected for this model. In particular, the power law model has been employed previously to predict whether the shear thinning or shear thickening based on n value (Kieferle et al., 2019) where $n < 1$ denoted that the samples are shear-thinning fluids (Sauer, Doehner, & Moraru, 2012). The apparent viscosity collected from the study was used to calculate the consistency coefficient (m) and flow behavior index (n). The Consistency coefficient and flow behavior index are the two parameters that were calculated based on the apparent viscosity based on the following formula:

Apparent viscosity is calculated by the equation assuming yield stress = 0,

$$\mu_{app=} - K\,\gamma^{n-1} \tag{3}$$

Were,

$\mu_{app}$ represents Apparent viscosity (Pa s).

$K$ represents the consistency coefficient (Pa $s^n$).

$\gamma$ represents shear rate ($s^{-1}$).

$n$ represents the flow behavior index.

Applying the log function to eq. (3),

$$\text{Log}\left(\mu_{app}\right) = \text{Log}\,(K) + (n-1)\,\text{Log}\,\gamma \tag{4}$$

Eq. (4) can be transformed to a line equation of y = ax + b where b represents intercept and a represents slope.

y – Log $(\mu_{app})$.

b – Log $(K)$ – intercept.

a - $(n\text{-}1)$ - slope.

x - Log $\gamma$.

The MCCs exhibited a shear thinning behavior with increased shear stress. Moreover, it is evident from the literature that MCC >7.5% casein concentration exhibited non-Newtonian or shear thinning behavior, which is on par with this study by Sauer et al. (2012). The casein concentration has been identified as a significant contributor previously to the viscosity characteristic of MCC (Solanki & Rizvi, 2001) but is also affected by the interference of other components like serum protein, lactose, minerals in MCC, with casein-casein interaction which can lead to reduced viscosity (Sauer et al., 2012). Also, it has been shown that the shear thinning behavior of MCC is influenced by composition, concentration, temperature, and storage time (Sauer et al., 2012; Solanki & Rizvi, 2001). The results from this study also confirmed this shear thinning behavior of MCC after the PEF treatment with $n < 1$. The result for the apparent viscosity of PEF-treated MCC (typical run) is shown in Fig. 1. The samples exhibited a shear stress thinning behavior at low shear rates, which differs from other ingredients where shear stress grows at a constant shear rate. Because all the MCC samples showed non-Newtonian behavior across the investigated range of shear rates,

**Table 3**
Temperature changes observed before and after PEF treatment to MCC.

| Runs | Temperature Input (°C) | Temperature output (°C) | ΔT(°C) |
|---|---|---|---|
| 1 | 45.00 | 46.11 | 1.11 |
| 2 | 45.00 | 45.00 | 0.00 |
| 3 | 15.00 | 23.28 | 8.28 |
| 4 | 30.00 | 30.00 | 0.00 |
| 5 | 30.00 | 30.56 | 0.56 |
| 6 | 45.00 | 45.00 | 0.00 |
| 7 | 15.00 | 21.67 | 6.67 |
| 8 | 15.00 | 21.67 | 6.67 |
| 9 | 45.00 | 45.00 | 0.00 |
| 10 | 30.00 | 31.67 | 1.67 |
| 11 | 15.00 | 20.01 | 5.00 |
| 12 | 15.00 | 35.83 | 12.50 |
| 13 | 30.00 | 32.78 | 2.78 |
| 14 | 45.00 | 47.17 | 2.17 |
| 15 | 30.00 | 31.61 | 1.61 |
| 16 | 30.00 | 30.56 | 0.56 |
| 17 | 30.00 | 31.39 | 1.39 |
| 18 | 30.00 | 32.22 | 2.22 |

**Table 4**
The face-centered central composite design and temperature difference after PEF treatment for MCC were generated using Stat Ease 360 software.

| Runs | Temperature (°C) | EFS (kV/cm) | Frequency (Hz) | Consistency coefficient, K (Pa $s^n$) |
|---|---|---|---|---|
| 1 | 45 | 12 | 165 | 865.59 |
| 2 | 45 | 20 | 30 | 2664.98 |
| 3 | 15 | 12 | 165 | 3809.31 |
| 4 | 30 | 4 | 165 | 1963.55 |
| 5 | 30 | 12 | 165 (Center) | 2316.87 |
| 6 | 45 | 4 | 30 | 1613.89 |
| 7 | 15 | 20 | 30 | 2749.28 |
| 8 | 15 | 4 | 30 | 2946.94 |
| 9 | 45 | 4 | 300 | 1781.77 |
| 10 | 30 | 20 | 165 | 1538.45 |
| 11 | 15 | 4 | 300 | 2995.77 |
| 12 | 15 | 20 | 300 | 2741.32 |
| 13 | 30 | 12 | 300 | 2565.07 |
| 14 | 45 | 20 | 300 | 3056.02 |
| 15 | 30 | 12 | 165 (Center) | 2348.72 |
| 16 | 30 | 12 | 30 | 2495.49 |
| 17 | 30 | 12 | 165 (Center) | 2537.74 |
| 18 | 30 | 12 | 165 (Center) | 2695.84 |
| 19 | 15 | 0 | 0 | 2787.28 |
| 20 | 30 | 0 | 0 | 2669.03 |
| 21 | 45 | 0 | 0 | 3011.36 |

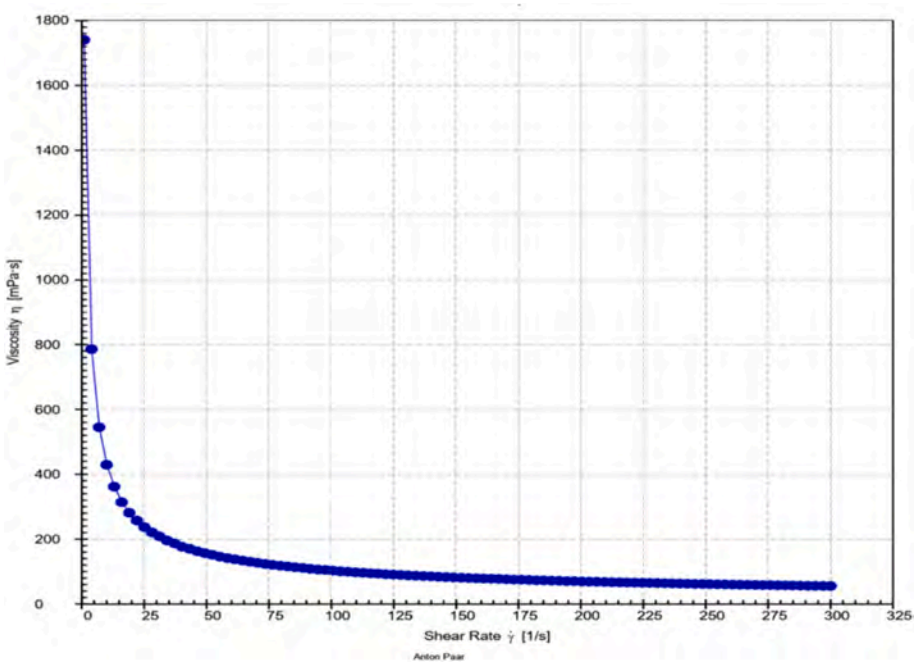


**Fig. 1.** Apparent viscosity of PEF-treated MCC (typical run).

the consistency coefficient was calculated keeping $n = 0.5$ constant (using the line equation, the average was calculated) and to keep the calculations consistent. Both m and n are considered functions of the protein content present in MCC.

Additionally, the apparent viscosity of MCC has also been noted to increase with increased concentration of casein due to beta-casein exclusion into the solution at lower temperatures (Sauer et al., 2012). For this reason, 12% MCC solutions were tested, and the concentration was kept constant to avoid variation in its apparent viscosity and to understand the effect of PEF on the viscosity characteristic of MCC. The apparent viscosity of MCC is also known to decrease with an increase in temperature. However, this study used 15–45 °C to test the viscosity behavior to avoid heat-induced treatments and minimize the structural effect of protein changes, which would indirectly impact the function.

### 3.3. Response surface analysis of viscosity

The effect of PEF processing parameters was analyzed for the minimum consistency coefficient of MCC as a reduction in consistency coefficient would indicate reduced viscosity. ANOVA was used to analyze the interaction terms' coefficients for MCC's consistency coefficient. The effect of process parameters was evaluated based on if the *p*-value obtained ($p < 0.05$) is statistically significant or insignificant, and finally, the model was assessed for significance. Compared with conventional and other heat, chemical, or enzymatic processing methods, PEF processing protects the protein functionality like gelation and viscosity more than the thermal treatments for dairy proteins (Sharma et al., 2016). PEF is also shown to improve energy and cost-saving in the dairy industry (Bermúdez-Aguirre, Yáñez, Dunne, Davies, & Barbosa-Cánovas, 2010).

#### 3.3.1. Interaction profiles of PEF processing parameters

Additionally, interaction profiles were studied with JMP pro® version 17 software. Fig. 2. indicates the interaction profiles for each response reviewed for optimization; the cross-over shows the interaction between processing parameters of PEF. The cross-over and curve in the interaction profiles indicate an interaction between two different parameters for the consistency coefficient under study, and the parallel line indicates less interaction or no interaction. From the Fig. 2. for the

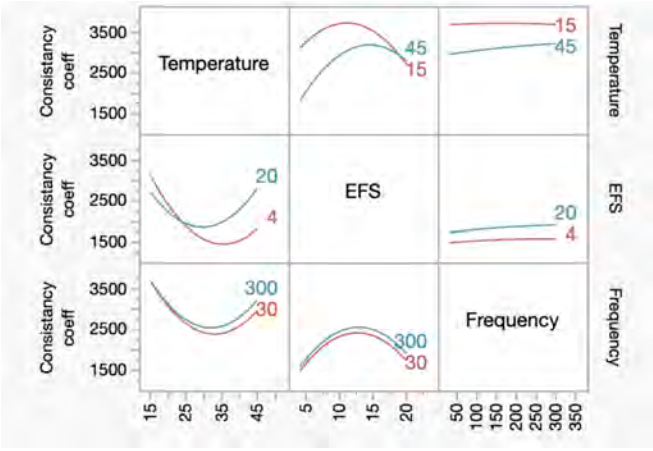


**Fig. 2.** Interaction profiler for consistency coefficient of PEF treated MCC from JMP pro 17®.

interaction profiler, it can be observed that the frequency factor has the most minor interaction profile, followed by temperature and EFS, which have the maximum interaction, which is also evident from ANOVA from Table 5. Additionally, the individual effect of temperature is highly significant at a 95% confidence interval with a *p*-value of 0.0112 (from Table 5), indicating that the temperature had the maximum effect on PEF treatment with MCC.

The plot temperature* EFS interaction shows that the effect on the consistency coefficient is relatively constant at higher levels of EFS and variable at lower levels of EFS. However, the effect on the consistency coefficient was higher at different temperature levels. This interaction is significant with a p-value of 0.0061(from Table 5), which is $p < 0.05$ at a 95% confidence interval. On the other hand, with frequency, the interaction profiles with EFS and temperature need to be more robust to prove an effect on the consistency coefficient of MCC.

#### 3.3.2. Response surface profiler and effects analysis

The 3D surface models shown in Fig. 3. can assist in concluding that

**Table 5**
Summary of effects for PEF treated MCC – Pre-Treatment and ANOVA for the fitted model.

| Source | Sum of squares | Df | Mean square | F-value | P-value | |
|---|---|---|---|---|---|---|
| **Model** | $4.774 \times 10^6$ | 9 | $5.305 \times 10^5$ | 8.25 | 0.0055* | *significant |
| A-Temperature | $7.511 \times 10^5$ | 1 | $7.511 \times 10^5$ | 11.69 | 0.0112* | |
| B-Electric field strength | $2.097 \times 10^5$ | 1 | $2.097 \times 10^5$ | 3.26 | 0.1138 | |
| C-Frequency | 44,805.62 | 1 | 44,805.62 | 0.6971 | 0.4313 | |
| AB | $9.643 \times 10^5$ | 1 | $9.643 \times 10^5$ | 15.00 | 0.0061* | |
| AC | 33,546.98 | 1 | 33,546.98 | 0.5220 | 0.4934 | |
| BC | 3459.87 | 1 | 3459.87 | 0.0538 | 0.8232 | |
| $A^2$ | $1.452 \times 10^6$ | 1 | $1.452 \times 10^6$ | 22.58 | 0.0021* | |
| $B^2$ | $1.486 \times 10^6$ | 1 | $1.486 \times 10^6$ | 23.12 | 0.0019* | |
| $C^2$ | 2241.95 | 1 | 2241.95 | 0.0349 | 0.8571 | |
| **Residual** | $4.499 \times 10^5$ | 7 | 64,270.81 | | | |
| Lack of Fit | $3.562 \times 10^5$ | 4 | 89,059.37 | 2.85 | 0.2078 | not significant |
| Pure Error | 93,658.18 | 3 | 31,219.39 | | | |
| **Cor Total** | $5.224 \times 10^6$ | 16 | | | | |

an increase in temperature had a maximum effect on the MCC consistency coefficient. At 45 °C temperature, 20 kV/cm electric field strengths, results showed a high consistency coefficient of 2664.98 Pa $s^n$. At 30 °C, it exhibits a more varied response; at 12 kV/cm, the consistency coefficient varies with frequency. The highest value was recorded when the frequency was 165 Hz at 2695.84 Pa $s^n$, indicating that specific frequency-temperature combinations influence the fluid's viscosity, which is also the center point in the experimental design.

Experiments conducted at center points represent the central points in the experimental design. These values remain crucial in understanding the reproducibility of the experiments in specific, well-defined conditions. At 20 kV/cm, the consistency coefficient was 1538.45 Pa $s^n$, indicating that higher EFS will be more effective at 30 °C. This also suggests that the interaction between temperature and EFS is crucial and not a linear relationship.

At 15 °C, the consistency coefficient is at its maximum at 12 kV/cm and 165 Hz to 3809.31 Pa $s^{n;}$ however, the consistency coefficient decreases at 20 kV/cm and 30 Hz. This suggests that viscosity is influenced by both temperature and frequency, with the electric field being a significant factor. Higher temperature and higher EFS correspond to an increase in the consistency coefficient. The results suggest a positive correlation between temperature and the consistency coefficient. Overall, across the temperature range, there is a trend of decrease or increase in consistency coefficient, emphasizing the synergistic effect of temperature and EFS on viscosity more. The temperature-dependent behavior also indicates that MCC viscosity is sensitive to changes in temperature. Combining high temperature, lower EFS, and low-frequency results in a lower consistency coefficient of 1613.89 Pa $s^n$, meaning reduced viscosity. This suggests that these conditions collectively contribute to an optimal state for maintaining the consistency coefficient.

At 20 kV/cm and 45 °C, the consistency coefficient was 2664.98 Pa $s^n$, significantly higher than at 12 kV/cm (865.59 Pa $s^n$). Reverse trends were observed at 30 °C, at 20 kV/cm; the consistency coefficient was lower (1538.45 Pa $s^n$) compared to 4 kV/cm at (1613.89 Pa $s^n$) and 12 kV/cm was 2348.72 Pa $s^n$. Also, at 30 °C, the consistency coefficient is higher at 12 kV/cm (2348.72 Pa $s^n$) than at 4 kV/cm (1963.55 Pa $s^n$). Similar trends were observed for 15 °C, suggesting that the effect of EFS is temperature-dependent. The influence of EFS is often interwoven with temperature, as observed in the experimental data, which also agrees with the model (Table 4 and Fig. 1.), where interaction between temperature and EFS is significant. Also, it is essential to note that at 30 °C, the effect of EFSis complex, with variations in consistency coefficients observed at different frequencies tested. Again, as indicated before, the temperature-EFS interaction is not straightforward and may depend on other factors. Although EFS may also interact with frequency to influence the viscosity profile, according to the model and data obtained, frequency does not significantly affect MCC's consistency coefficient. At 30 °C, the consistency coefficient varies with frequency at 4 kV/cm and 12 kV/cm. This suggests that the impact of EFS on consistency is modulated by the frequency of the applied electric field but is not significant enough to cause a major change according to the model generated.

Analyzing Table 4 runs 4, 6, and 9 produced a lower consistency coefficient with a minimum of 1613.89 Pa $s^n$. Similarly, runs 3, 8, and 11 had a higher consistency coefficient with a maximum of 3809.31 Pa $s^n$. The reduced consistency coefficient could be due to changes in the protein backbone structure caused by PEF treatment, for the PEF treatment conditions that have consistently observed minimum viscosity might be caused by partial denaturation and optimal treatment leading to partial exposure of certain groups on the surface of the casein protein. Previous research has shown that PEF is also known to increase the volume of the casein micelles and create pores. The outcomes signify that PEF processing reduces MCC's viscosity in less time than control samples without PEF treatment. PEF can be used as a treatment to modify the viscosity in a short period of 26 m seconds.

To understand the model and interaction profiles, the data was thoroughly analyzed using JMP for outliers. Run 1 (temperature 45 °C, EFS 12 kV/cm and 165 Hz) with a consistency coefficient of 865.59 Pa $s^n$ was identified as an outlier. The value obtained for the consistency coefficient is way less than all the other experimental runs, potentially due to environmental factors, time or temperature-dependent changes, or excessive treatment of MCC. In this study, temperature controls were used to eliminate any changes to the samples due to changes in temperature during the treatment. Therefore, viscosity analysis was still performed at 25 °C; hence, the effect of temperature on modifying the viscosity was not considered.

Having EFS at 4 kV/cm for both runs 6 and 9 at a temperature of 45 °C and runs 8 and 11 at a temperature of 15 °C and varying frequency from 30 to 300 Hz suggests that an increase in frequency leads to a maximum of 2995.77 Pa $s^n$ and a drop in frequency provided a minimum of 1613.89 Pa $s^n$. Similarly, at the same EFS, runs 6 & 8 and 9 & 11 with varying temperatures from 15 °C to 45 °C led to a decrease in consistency coefficient despite an increase in frequency. At 12 kV/cm and 30 °C, varying the frequency from 30 to 165 Hz increased the consistency coefficient to a maximum of 2695.84 Pa sn, and further increasing to 300 Hz decreased the consistency coefficient to 2565.07 Pa $s^n$. Compared to the runs at mid-temperature and EFS, increasing the frequency to a certain extent had a maximum and then dropped when the frequency was extreme. At 20 kV/cm and lower frequency, the increase in temperature led to a decrease in consistency coefficient to 2664.98 Pa $s^n$ (Run 2 and 7). However, increasing the frequency to 300 Hz with the same EFS (Run 12 & 14) leads to an increase in consistency coefficient despite the rise in the temperature. This might be caused by a combined effect of all the three parameters. Run 14 accounts for the highest conditions in the experimental run, with the highest consistency coefficient of 3056.02 Pa $s^n$. With runs 7 and 12, at 20 kV/cm and a lower temperature of 15 °C, the consistency coefficient relatively remains constant

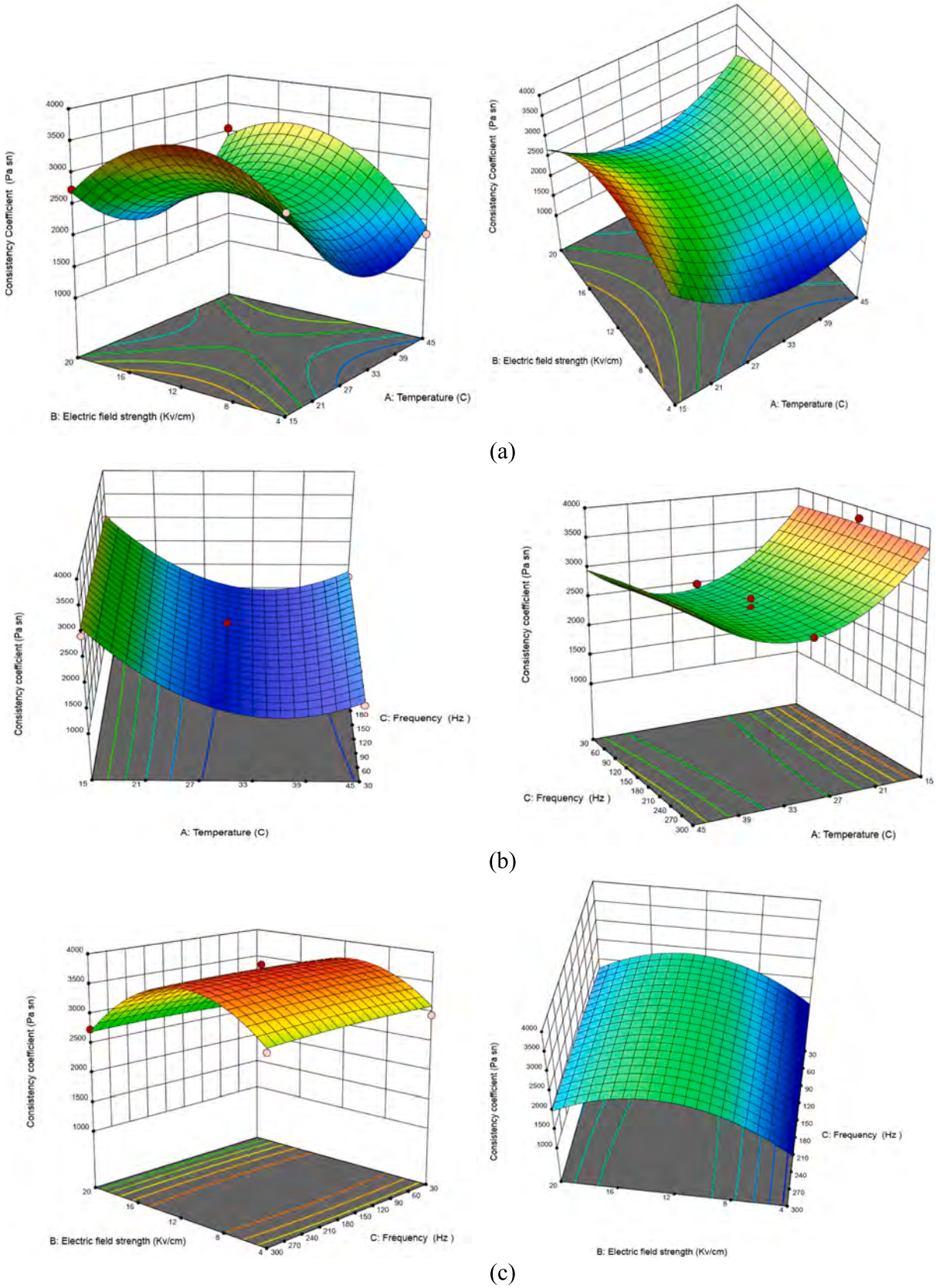


**Fig. 3.** 3D surface plots showing the mutual effects of (a) temperature and EFS, (b) temperature and frequency, and (c) electric field intensity and frequency for consistency coefficient of PEF treated MCC from Stat-Ease 360®.

with an increase in frequency. However, it is notable that the mid-temperature range of 30 °C mid-frequency of 165 Hz, and higher EFS of 20 kV/cm (Run 10) minimizes the consistency coefficient to 1538.45 Pa sn, which is also indicative that optimizing the PEF processing parameters could potentially assist in reducing the viscosity of MCC. Overall, alteration to the temperature and EFS was concluded to have a maximum effect on MCC viscosity, and a minor role with the frequency was observed.

The viscosity characteristic of dairy protein in solution is affected by concentration, size, surface properties, and interactions with other protein particles (Ranadheera et al., 2019). The result from this study shows that treatment with a PEF could lead to changes in the viscosity of MCC. These findings follow a study by (Xiang, 2008), where changes in apparent viscosity and shear stress were observed in skim milk after PEF treatment. Similar results have been reported to have reduced viscosity of milk (Xiang et al., 2011) and changes in apparent viscosity in soy milk (Bob, Ngadi, Gachovska, & Simpson, 2007). PEF processing has been known to ionize certain chemical groups of the protein backbone and cause changes in the secondary structure (Zhao, Tang, Lu, Chen, & Li, 2014). Depending on the EFS applied, PEF could have a positive or negative effect (Zhao et al., 2014). Studies state that the increase in hydrophobic amino acids from the inner core (Yu, Ngadi, & Raghavan, 2009) of protein structure could make the protein more flexible and hence change the function of the protein (Mirmoghtadaie, Shojaee Aliabadi, & Hosseini, 2016) and in this study is viscosity. Therefore, PEF treatment leads to conformational changes in the protein structure by modifying the ionic interactions between proteins (Yu et al., 2009).

### 3.4. Protein profiling analysis with SDS

The molecular weight distribution of PEF-treated MCC was characterized using SDS-PAGE. The SDS-PAGE analysis provides an understanding of inherent protein molecular weight distribution changes or if there is any aggregation of proteins. The electropherogram for MCC control and PEF-treated MCC samples are illustrated in Fig. 4. Fig. 4. represents the SDS PAGE profile of PEF-treated MCC samples under both reducing and non-reducing conditions. From the electropherogram, between the control and treated samples, they displayed bands of BSA, $\alpha_s$-CN, and β-CN.

The proteins were recorded in the range of 10 to 250 kDa. The electrophoretic patterns also represented a mix of low and high-molecular-weight proteins. The MW patterns from PEF-treated MCC showed no intensity changes in the bands after the PEF treatment. The present MW pattern findings conform with the results from Zhang et al. (2018). Similar results were reported for no changes in molecular weight distribution for whey protein isolate (Taha et al., 2022) and milk protein isolate treated with PEF (Raghunath et al., 2024). Based on the results, the milk protein fractions were similar to the literature, and the bands obtained were in the range (Zhang et al., 2018).

This analysis has demonstrated that the primary structure of the MCC were unaffected by the PEF treatment. The gels also show di-sulfide linkages aggregated at the stacking region of reducing gels (Havea, 2006). Both the reducing and the non-reducing gels show an apparent visual confirmation of protein bands such as αs casein, β casein, κ casein, β-lactoglobulin and α-lactalbumin (Tari, Gaygadzhiev, Guri, & Wright, 2021). This also suggests that recovering the protein fractions as monomeric protein is possible, redefining that primary protein fractions were not lost during the PEF treatment (Anema, Pinder, Hunter, & Hemar, 2006). Overall, the SDS-PAGE analysis justified that no significant changes in the MW distribution of MCC were observed due to PEF treatment.

### 3.5. Flow behavior analysis

The results of the flowability analysis of MCC are represented in terms of avalanche energy. According to the analytical method, the lower the avalanche energy correlated to the sample's increased or easy flow behavior. PEF-treated MCC had a mean avalanche energy range of 63.35 kJ/kg (control) - 38.47 kJ/kg. Overall, when comparing with the controls, the avalanche energy for PEF-treated MCC, there was a minor difference; however, it was not significant to be analyzed as a model. Protein powders like MCC are used as ingredients in various applications as the demand for high-protein powders in the market keeps increasing (Mahadev & Meena, 2020). MCCs form more casein-casein interactions due to spray drying and dipole effects, altering the surface composition (Mahadev & Meena, 2020). This will, in turn, lead to the aggregation of protein powders during the mixing process and make the MCC difficult to flow. The flow behavior of these MCCs is also highly influenced by the particle size of the MCC (Crowley et al., 2014; Fitzpatrick, Iqbal, Delaney, Twomey, & Keogh, 2004) and the composition (Fitzpatrick et al., 2004). PEF is known to cause changes to the particle size of the powder, and a decrease in particle sizes will have increased flow properties, which was also observed as part of the particle size analysis from Table 6. However, the changes are not significant. In general, high protein powders like MCC tend to have higher protein-protein interactions and protein–causing interactions due to the absence of lactose (Mahadev & Meena, 2020), leading to higher aggregation and particle size of the MCC samples. Thus, pre-treatment with PEF for MCC was beneficial to improving viscosity at 12%, but there was no significant model to predict the flow behavior pattern of PEF-treated MCC. A possible reason for this could be the initial lower solids content (12%) as a raw material for the PEF treatment, which may not result in a significant difference in spray drying efficiency. Conducting further research to understand how increasing the total solids content might affect flow behavior could help determine if there are any significant differences in the system.

### 3.6. Powder particle size analysis

The particle size analysis of powders was analyzed. Values are represented as D(v,0.1) representing particle size below 10% of the powder volume, D (v,0.5) representing particle size below which 50% of the volume exists, and D(v,0.9) representing particle size below which 90% of the material volume exists along with the mean particle size in Table 6. From the table, the mean particle size for all the MCC samples, including the controls, was similar, with minor differences. The largest median particle size MCC was observed to be 45.827 μm, and the minimum was found to be 36.638 μm. MCC samples with PEF treatment had similar particle size distribution compared to the sample controls from runs 19, 20, and 21. The mean particle size of MCC from PEF-treated samples was 36–40 μm, and the medium particle size was 30–34 μm. The minimum differences in the particle size of the powders could be attributed to spray dryer efficiency, consistency, electric field-induced particle size agglomeration, or reduction in the process. The TS content from the raw material could affect the particle size of the spray drying process (Keogh, Murray, & O'Kennedy, 2003) in which case it was constant at 12%. From the literature, the MCC particle size was d10 = 29.44 μm, d50 = 82.46 μm, and d90 = 110.3 μm (Song et al., 2022). The differences in the particle size between samples could be due to various environmental factors, total solids, concentration of the solution, atomizer speed used for the drying process, and the dryer's efficiency. Most of the d90 for PEF-treated MCC ranges between 70 and 74 μm and d50 at 30–33 μm, which is a ~ 30% decrease from what is seen in the literature. The particle size analysis provided an understanding that there is a minor difference in particle size compared with the temperature control samples and increased surface area contact. The reduced particle size increased flowability because the surface area increased and provided a more cohesive flow of the powder particles (Fitzpatrick et al., 2004; Song et al., 2022). Overall, the particle size analysis indicated that the samples under PEF treatment would have a potentially minor difference in particle size for MCC; however, it is not significant enough to provide a model prediction.

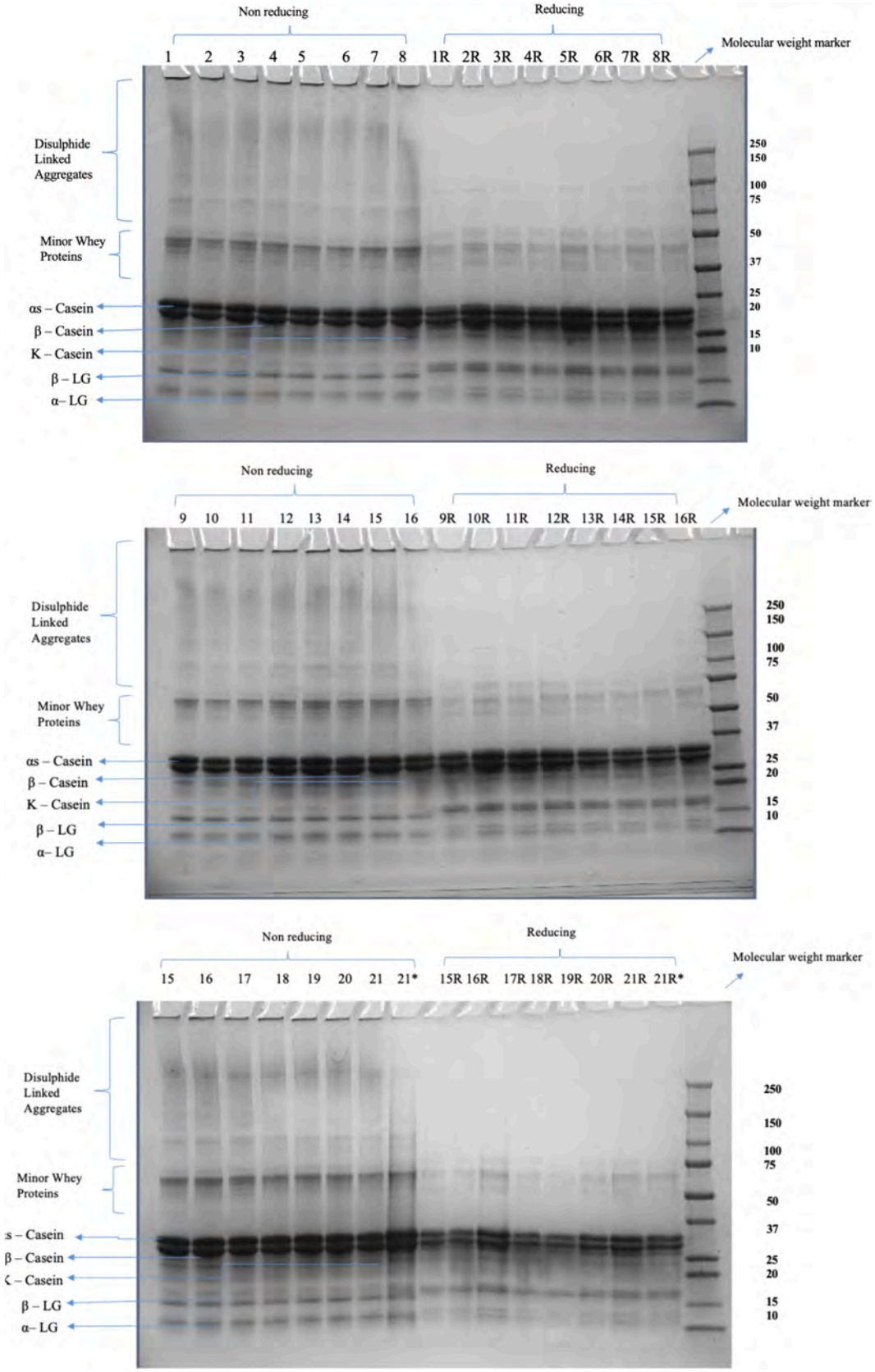


**Fig. 4.** SDS analysis of both reducing and non-reducing conditions of PEF-treated MCC. The numbers on the top of the electropherogram correspond to the runs listed in Table 4, and R represents reducing conditions under beta-mercaptoethanol. The numbers on top represent runs from Table 4 and numbers on the right represent the molecular weight marker in kDa.

**Table 6**
Average particle size analysis of reconstituted MCC (PEF treatment) and average flow behavior represented by mean avalanche energy after PEF treatment of MCC.

| Runs | Mean | Median | Mode | Std Dev | D(v,0.1) | D(v,0.9) | D(v,0.5) | Mean of avalanche energy (Mean ± SD) kJ/kg |
|---|---|---|---|---|---|---|---|---|
| 1 | 40.559 | 34.802 | 41.899 | 26.302 | 14.472 | 73.048 | 34.802 | 53 ± 8.09 |
| 2 | 39.818 | 32.075 | 41.997 | 30.207 | 12.942 | 74.226 | 32.075 | 51.5 ± 1.22 |
| 3 | 39.332 | 32.932 | 41.926 | 27.139 | 13.576 | 72.073 | 32.932 | 45.33 ± 2.05 |
| 4 | 39.068 | 32.72 | 36.698 | 26.441 | 14.063 | 70.957 | 32.72 | 61.97 ± 3.26 |
| 5 | 37.892 | 30.613 | 42.026 | 26.761 | 12.605 | 72.34 | 30.613 | 52.1 ± 2.62 |
| 6 | 39.689 | 33.412 | 41.939 | 26.736 | 13.594 | 73.109 | 33.412 | 45.76 ± 4.39 |
| 7 | 41.673 | 35.000 | 48.195 | 28.893 | 13.085 | 78.296 | 35 | 50.93 ± 1.16 |
| 8 | 38.853 | 32.939 | 41.969 | 25.341 | 13.528 | 71.623 | 32.939 | 44.27 ± 3.26 |
| 9 | 37.917 | 30.907 | 41.955 | 26.955 | 12.499 | 71.78 | 30.907 | 36.43 ± 2.99 |
| 10 | 39.109 | 31.233 | 42.073 | 28.587 | 12.742 | 74.724 | 31.233 | 44.7 ± 4.16 |
| 11 | 39.579 | 33.241 | 48.035 | 26.517 | 12.725 | 74.841 | 33.241 | 44.7 ± 8.03 |
| 12 | 37.61 | 31.707 | 47.834 | 25.018 | 12.283 | 70.766 | 31.707 | 38.47 ± 2.47 |
| 13 | 38.683 | 32.012 | 41.971 | 26.974 | 13.17 | 71.913 | 32.012 | 48.57 ± 3.38 |
| 14 | 36.638 | 31.531 | 36.703 | 22.554 | 13.5 | 66.336 | 31.531 | 49.37 ± 6.81 |
| 15 | 39.103 | 32.500 | 41.925 | 27.372 | 13.309 | 72.177 | 32.5 | 46.5 ± 2.26 |
| 16 | 38.604 | 31.451 | 41.886 | 27.647 | 13.265 | 71.919 | 31.451 | 49.37 ± 19.56 |
| 17 | 40.639 | 33.705 | 48.118 | 28.193 | 12.954 | 76.838 | 33.705 | 53.4 ± 6.9 |
| 18 | 45.827 | 41.583 | 48.36 | 28.084 | 14.982 | 80.881 | 41.583 | 39.73 ± 4.12 |
| 19 | 39.647 | 32.949 | 42.026 | 27.379 | 13.425 | 73.612 | 32.945 | 63.35 ± 12.35 |
| 20 | 39.197 | 33.317 | 39.336 | 25.565 | 14.081 | 71.15 | 33.317 | 47.35 ± 5.35 |
| 21 | 39.526 | 33.267 | 42.009 | 27.087 | 13.456 | 72.552 | 33.267 | 42.16 ± 0.94 |

Footnote: Values represent the particle size of MCC as average calculations for triplicate sample analysis. D(v,0.1) represents particle size below 10% of the powder volume, D(v,0.5) represents the particle size of powders below 50% of the volume, and D(v,0.9) represents particle size below 90% of the material volume.

### 3.7. Optimization of process variables

The experimental dataset observed that customization of MCC for reduced viscosity can be achieved through the PEF as a pre-treatment during manufacturing. Table 5 shows ANOVA results, and since $p < 0.05$, the model proves a significant fit, and the lack of fit is not significant.

The experimental data (Table 4) was fitted into a quadratic polynomial given by,

$$\textbf{Consistency Coefficient}\,(\mathrm{Pa\,s^n})\,\text{for MCC} = 2518.28 - 300.45A + 144.81B + 66.94C + 347.18AB + 64.76AC + 20.80BCE + 903.60A^2 - 810.77B^2 - 31.49C^2. \quad (5)$$

*A, B, and C* represent temperature, electric field strength, and frequency; AB, BC, and AC represent interaction terms; and $A^2$, $B^2$, *and* $C^2$ represent quadratic terms in eq. (5). The data followed a quadratic model with $R^2$ of 0.91 and adjusted $R^2$ of 0.80, satisfying conditions for a good model fit. This equation can predict the consistency coefficient of PEF-treated MCC. The 3D surface plots (Fig. 3.) for processing parameters of PEF have been represented with the independent variables that affected the consistency coefficient. Based on the desirability value, it can be concluded that the optimized condition will agree with the predicted value 99% of the time when the experiment is conducted.

This research observed that PEF treatment can increase or decrease the consistency coefficient depending on the treatment conditions applied. Thus, optimizing the PEF treatment to provide a reduced viscosity profile is very trivial in preventing excessive treatment and denaturation of the protein. The optimized conditions for minimum consistency coefficient/reduced viscosity of PEF-treated MCC were obtained using a numerical optimization procedure in DOE. They are as follows: Temperature at 34.7 °C, EFS at 4.26 kV/cm, and frequency at 62.76 Hz. The optimized model has a consistency coefficient of 1440.57 Pa $s^n$ and desirability of 1.0, thereby minimizing the consistency coefficient. The PEF treatment using optimized process conditions resulted in a consistency coefficient that is 48.31% less than that of the 15 °C control (2787.28 Pa $s^n$), 46.02% less than that of the 30 °C control (2669.03 Pa sn), and 52.16% less than that of 45 °C control (3011.36 Pa $s^n$). With maximum viscosity improvement, using PEF will allow MCC to be used as an ingredient in many new product applications, including bars, supplements, and medical nutrition.

Thus, having mid-temperature, lower frequency, and mid-EFS would assist in producing MCC with enhanced. Since ranges were selected for the study, the optimized results will only be conclusive for these parameters. From runs 12 and 3, increasing the system's frequency and EFS might not be ideal since the increase causes a rise in temperature of a few degrees, and based on the current study, the maximum is about 12 °C.

## 4. Conclusions

In conclusion, this study analyzed and optimized the effect of the PEF on the viscosity of MCC. For many practical applications of methods, it has always been helpful to have these mathematically optimized models to accurately predict the viscosity behavior of MCC and how it can be optimized and reduced. One such method, pulsed electric field, was studied. The study showed that PEF could be used as a treatment to reduce the viscosity of MCC. RSM indicated that the interaction effect between temperature and EFS, as well as temperature as an individual factor, played a significant role in optimizing the conditions for the treatment. The optimized conditions for PEF to produce MCC with a low viscosity profile include Temperature at 34.7 °C, EFS at 4.26 kV/cm and frequency at 62.76 Hz with 1440.57 Pa $s^n$ which was 46% less than control at 30 °C. The power law model indicated that the PEF-treated MCC samples exhibited shear thinning behavior. SDS protein profiling of PEF-treated MCC displayed that the primary structure of the proteins is still maintained and observed no changes.

On the other hand, the particle size analysis showed a slight reduction in the particle size for PEF-treated samples compared to the control, indicating a change in aggregation of molecules and improved flowability of the MCC powders with PEF treatment however the data was not fitted to model to be considered significant. Overall, this research supports the idea that reduced viscosity following the PEF treatment is achievable. Further research might explore the effect of other processing parameters and the different TS and functional properties of MCC. The optimized model thus developed will provide the dairy industry with low-viscosity MCC, which would be necessary for new product innovations.

## Funding

This project has received funding from National Dairy Council, USA.

## CRediT authorship contribution statement

**Sonali Raghunath:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tonya Schoenfuss:** Supervision, Funding acquisition, Conceptualization. **Kumar Mallikarjunan:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sonali Raghunath, Tonya C Schoenfuss, Kumar Mallikarjunan has patent processing on SYSTEMS AND METHODS FOR MODIFYING DAIRY PROTEIN FUNCTIONALITY pending to Regents of the University of Minnesota (US Utility Patent Application No. 18/649,533). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgments

The authors thank Dr. Prafulla Salunke from the Department of Dairy Science, South Dakota State University for the viscosity analysis. Midwest Dairy Research Center provided an opportunity to present the research and the authors thank National Dairy Council for funding this project. This research was made possible with MnDrive Graduate Student Professional Development award and Tarleton Graduate assistantship from the Graduate School of the University of Minnesota.

## References

Anema, S. G., Pinder, D. N., Hunter, R. J., & Hemar, Y. (2006). Effects of storage temperature on the solubility of milk protein concentrate (MPC85). *Food Hydrocolloids, 20*(2–3), 386–393. https://doi.org/10.1016/J.FOODHYD.2005.03.015

Beckman, S. L., Zulewska, J., Newbold, M., & Barbano, D. M. (2010). Production efficiency of micellar casein concentrate using polymeric spiral-wound microfiltration membranes. *Journal of Dairy Science, 93*(10), 4506–4517. https://doi.org/10.3168/JDS.2010-3261

Bekard, I., & Dunstan, D. E. (2013). Electric field-induced changes in protein conformation. *Soft Matter, 10*(3), 431–437. https://doi.org/10.1039/C3SM52653D

Beliciu, C. M., Sauer, A., & Moraru, C. I. (2012). The effect of commercial sterilization regimens on micellar casein concentrates. *Journal of Dairy Science, 95*(10), 5510–5526. https://doi.org/10.3168/JDS.2011-4875

Bermúdez-Aguirre, D., Yáñez, J. A., Dunne, C. P., Davies, N. M., & Barbosa-Cánovas, G. V. (2010). Study of strawberry flavored milk under pulsed electric field processing. *Food Research International, 43*(8), 2201–2207. https://doi.org/10.1016/j.foodres.2010.07.021

Bob, X., Ngadi, M., Gachovska, T., & Simpson, B. K. (2007). *Pulse electric field treatment effects on rheological properties and color of soy milk. 2007 ASABE annual international meeting, technical papers, 11 BOOK(January 2007)*. https://doi.org/10.13031/2013.23290

Crowley, S. V., Gazi, I., Kelly, A. L., Huppertz, T., & O'Mahony, J. A. (2014). Influence of protein concentration on the physical characteristics and flow properties of milk protein concentrate powders. *Journal of Food Engineering, 135*, 31–38. https://doi.org/10.1016/J.JFOODENG.2014.03.005

Dunn, M., Barbano, D. M., & Drake, M. A. (2021). Viscosity changes and gel formation during storage of liquid micellar casein concentrates. *Journal of Dairy Science, 104*(12), 12263–12273. https://doi.org/10.3168/JDS.2021-20658

Farooq, Z. (2019). Study of thermal and hydrolytic denaturation of casein (αs1) using differential scanning calorimetry (DSC). *Journal of Animal and Plant Sciences, 29*(1), 141–148.

Fitzpatrick, J. J., Iqbal, T., Delaney, C., Twomey, T., & Keogh, M. K. (2004). Effect of powder properties and storage conditions on the flowability of milk powders with different fat contents. *Journal of Food Engineering, 64*(4), 435–444. https://doi.org/10.1016/J.JFOODENG.2003.11.011

Floury, J., Grosset, N., Leconte, N., Pasco, M., Madec, M. N., & Jeantet, R. (2006). Continuous raw skim milk processing by pulsed electric field at non-lethal temperature: Effect on microbial inactivation and functional properties. *Lait, 86*(1), 43–57. https://doi.org/10.1051/lait:2005039

Grossbier, D. (2016). *Manufacture, concentration, and functionality of micellar casein concentrate*. South Dakota State University.

Hammam, A. R. A., Kapoor, R., & Metzger, L. E. (2023). Manufacture of a novel cultured micellar casein concentrate ingredient for emulsifying salt–free process cheese products applications. *Journal of Dairy Science, 106*(5), 3137–3154. https://doi.org/10.3168/JDS.2022-22652

Hammam, A. R. A., Martínez-Monteagudo, S. I., & Metzger, L. E. (2021). Progress in micellar casein concentrate: Production and applications. *Comprehensive Reviews in Food Science and Food Safety, 20*(5), 4426–4449. https://doi.org/10.1111/1541-4337.12795

Hammam, A. R. A., & Metzger, L. E. (2023). Characteristics of imitation mozzarella cheese manufactured without emulsifying salts using a combination of culture-based acid curd and micellar casein concentrate. *Food Science & Nutrition, 11*(8), 4616–4624. https://doi.org/10.1002/FSN3.3424

Havea, P. (2006). Protein interactions in milk protein concentrate powders. *International Dairy Journal, 16*(5), 415–422. https://doi.org/10.1016/J.IDAIRYJ.2005.06.005

Hemar, Y., Augustin, M. A., Cheng, L. J., Sanguansri, P., Swiergon, P., & Wan, J. (2011). The effect of pulsed electric field processing on particle size and viscosity of milk and milk concentrates. *Milchwissenschaft, 66*(2), 126–128.

Huppertz, T. (2013). Chemistry of the caseins. In , *Vol. 1 A. Advanced dairy chemistry* (pp. 135–160). Springer US. https://doi.org/10.1007/978-1-4614-4714-6_4.

Hurt, E., Zulewska, J., Newbold, M., & Barbano, D. M. (2010). Micellar casein concentrate production with a 3X, 3-stage, uniform transmembrane pressure ceramic membrane process at 50°C1. *Journal of Dairy Science, 93*(12), 5588–5600. https://doi.org/10.3168/jds.2010-3169

Keogh, M. K., Murray, C. A., & O'Kennedy, B. T. (2003). Effects of ultrafiltration of whole milk on some properties of spray-dried milk powders. *International Dairy Journal, 13*(12), 995–1002. https://doi.org/10.1016/S0958-6946(03)00123-7

Kieferle, I., Hiller, K., Kulozik, U., & Germann, N. (2019). Rheological properties of fresh and reconstituted milk protein concentrates under standard and processing conditions. *Journal of Colloid and Interface Science, 537*, 458–464. https://doi.org/10.1016/J.JCIS.2018.11.048

Lagrange, V., Whitsett, D., & Burris, C. (2015). Global market for dairy proteins. *Journal of Food Science, 80*(S1), A16–A22. https://doi.org/10.1111/1750-3841.12801

Li, H., Zhao, T., Li, H., & Yu, J. (2021). Effect of heat treatment on the property, structure, and aggregation of skim Milk proteins. *Frontiers in Nutrition, 8*, Article 714869. https://doi.org/10.3389/FNUT.2021.714869/BIBTEX

Mahadev, G. M., & Meena, G. S. (2020). Milk protein concentrates 80: Does composition of buffalo milk matter for its poor functionality? *LWT, 131*, Article 109652. https://doi.org/10.1016/J.LWT.2020.109652

McMahon, D. J., & Oommen, B. S. (2013). Casein micelle structure, functions, and interactions. In *Advanced dairy chemistry: Volume 1A: Proteins: Basic aspects* (4th ed., pp. 185–209). Springer US. https://doi.org/10.1007/978-1-4614-4714-6_6.

McSweeney, P. L. H., & Fox, P. F. (2013). Advanced dairy chemistry: Volume 1A: Proteins: Basic aspects, 4th edition. In *In advanced dairy chemistry: Volume 1A: Proteins: Basic aspects, 4th edition*. Springer: US. https://doi.org/10.1007/978-1-4614-4714-6.

Mirmoghtadaie, L., Shojaee Aliabadi, S., & Hosseini, S. M. (2016). Recent approaches in physical modification of protein functionality. *Food Chemistry, 199*, 619–627. https://doi.org/10.1016/j.foodchem.2015.12.067

Misawa, N., Barbano, D. M., & Drake, M. A. (2016). Influence of casein as a percentage of true protein and protein level on color and texture of milk containing 1 and 2% fat. *Journal of Dairy Science, 99*(7), 5284–5304. https://doi.org/10.3168/JDS.2016-10846

Nasser, S., Jeantet, R., De-Sa-Peixoto, P., Ronse, G., Nuns, N., Pourpoint, F., … Delaplace, G. (2017a). Microstructure evolution of micellar casein powder upon aging: Consequences on rehydration dynamics. *Journal of Food Engineering, 206*, 57–66. https://doi.org/10.1016/J.JFOODENG.2017.03.004

Nasser, S., Moreau, A., Jeantet, R., Hédoux, A., & Delaplace, G. (2017b). Influence of storage conditions on the functional properties of micellar casein powder. *Food and Bioproducts Processing, 106*, 181–192. https://doi.org/10.1016/J.FBP.2017.09.004

Nelson, B. K., & Barbano, D. M. (2005). Yield and aging of Cheddar cheeses manufactured from milks with different milk serum protein contents. *Journal of Dairy Science, 88*(12), 4183–4194. https://doi.org/10.3168/jds.S0022-0302(05)73104-0

O' Mahony, J. A., & Fox, P. F. (2013). Milk proteins: Introduction and historical aspects. In *Advanced dairy chemistry: Volume 1A: Proteins: Basic aspects* (4th ed., pp. 43–85). Springer US. https://doi.org/10.1007/978-1-4614-4714-6_2.

Pellegrino, L., Masotti, F., Cattaneo, S., Hogenboom, J. A., & De Noni, I. (2013). Nutritional quality of milk proteins. In *Advanced dairy chemistry: Volume 1A: Proteins: Basic aspects* (4th ed., pp. 515–538). Springer US. https://doi.org/10.1007/978-1-4614-4714-6_16.

Raghunath, S., Mallikarjunan, P. K., & Schoenfuss, T. C. (2021). *Optimization and effect of pulsed electric field processing parameters on insolubility index of reconstituted milk protein concentrate 85 (MPC85). 2021 ASABE annual international virtual meeting, 1*.

Raghunath, S., Mallikarjunan, P. K., & Schoenfuss, T. C. (2024). *Optimizing pulsed electric field processing for improving functionality of reconstituted milk protein concentrate 85*

*(MPC85) with response surface methodology* [Unpublished doctoral dissertation]. University of Minnesota

Ranadheera, C. S., Liyanaarachchi, W. S., Dissanayake, M., Chandrapala, J., Huppertz, T., & Vasiljevic, T. (2019). Impact of shear and pH on properties of casein micelles in milk protein concentrate. *LWT, 108*, 370–376. https://doi.org/10.1016/J.LWT.2019.03.090

Rodrigues, R. M., Avelar, Z., Vicente, A. A., Petersen, S. B., & Pereira, R. N. (2020). Influence of moderate electric fields in β-lactoglobulin thermal unfolding and interactions. *Food Chemistry, 304*, Article 125442. https://doi.org/10.1016/J.FOODCHEM.2019.125442

Sauer, A., Doehner, I., & Moraru, C. I. (2012). Steady shear rheological properties of micellar casein concentrates obtained by membrane filtration as a function of shear rate, concentration, and temperature. *Journal of Dairy Science, 95*(10), 5569–5579. https://doi.org/10.3168/jds.2012-5501

Schulnies, F., Höhme, L., & Kleinschmidt, T. (2023). Ultrasonication of micellar casein concentrate to reduce viscosity — Role of undissolved material. *Foods, 12*, 4519.

Sharma, P., Oey, I., & Everett, D. W. (2016). Thermal properties of milk fat, xanthine oxidase, caseins and whey proteins in pulsed electric field-treated bovine whole milk. *Food Chemistry, 207*, 34–42. https://doi.org/10.1016/j.foodchem.2016.03.076

Solanki, G., & Rizvi, S. S. H. (2001). Physico-chemical properties of skim Milk Retentates from microfiltration. *Journal of Dairy Science, 84*(11), 2381–2391. https://doi.org/10.3168/JDS.S0022-0302(01)74687-5

Song, B., Yao, P., Zhang, Y., Pang, X., Zhang, S., & Lv, J. (2022). Prior to spray drying, ultrasound pretreatment improves the flowability and water sorption properties of micellar casein concentrate. *Ultrasonics Sonochemistry, 87*, Article 106049. https://doi.org/10.1016/J.ULTSONCH.2022.106049

Sui, Q., Roginski, H., Williams, R. P. W., Versteeg, C., & Wan, J. (2011). Effect of pulsed electric field and thermal treatment on the physicochemical and functional properties of whey protein isolate. *International Dairy Journal, 21*(4), 206–213. https://doi.org/10.1016/J.IDAIRYJ.2010.11.001

Syed, Q. A., Ishaq, A., Rahman, U. U., Aslam, S., & Shukat, R. (2017). Pulsed electric field Technology in Food Preservation: A review. *Journal of Nutritional Health & Food Engineering, 6*(6), 168–172. https://doi.org/10.15406/jnhfe.2017.06.00219

Taha, A., Casanova, F., Šimonis, P., Stankevič, V., Gomaa, M. A. E., & Stirkė, A. (2022). Pulsed electric field: Fundamentals and effects on the structural and techno-functional properties of dairy and plant proteins. *Foods, 11*(11). https://doi.org/10.3390/FOODS11111556

Taha, A., Casanova, F., Talaikis, M., Stankevič, V., Žurauskienė, N., Šimonis, P., … Stirkė, A. (2023). Effects of pulsed electric field on the physicochemical and structural properties of micellar casein. *Polymers, 15*(15). https://doi.org/10.3390/POLYM15153311

Tari, N. R., Gaygadzhiev, Z., Guri, A., & Wright, A. (2021). Effect of pH and heat treatment conditions on physicochemical and acid gelation properties of liquid milk protein concentrate. *Journal of Dairy Science, 104*(6), 6609–6619. https://doi.org/10.3168/jds.2020-19355

Walter, J., Greenberg, Y., Sriramarao, P., & Ismail, B. P. (2016). Limited hydrolysis combined with controlled Maillard-induced glycation does not reduce immunoreactivity of soy protein for all sera tested. *Food Chemistry, 213*, 742–752. https://doi.org/10.1016/j.foodchem.2016.07.012

Xiang, B. Y. (2008). *Effects of pulsed electric fields on structural modification and rheological properties for selected food proteins* (issue 1) [McGill University]. http://fipak.areeo.ac.ir/site/catalogue/18577717.

Xiang, B. Y., Simpson, M. V., Ngadi, M. O., & Simpson, B. K. (2011). Flow behaviour and viscosity of reconstituted skimmed milk treated with pulsed electric field. *Biosystems Engineering, 109*(3), 228–234. https://doi.org/10.1016/j.biosystemseng.2011.04.004

Yu, L. J., Ngadi, M., & Raghavan, G. S. V. (2009). Effect of temperature and pulsed electric field treatment on rennet coagulation properties of milk. *Journal of Food Engineering, 95*(1), 115–118. https://doi.org/10.1016/j.jfoodeng.2009.04.013

Zhang, S., Chen, J., Pang, X., Lu, J., Yue, M., Liu, L., & Lv, J. (2018). Pilot scale production of micellar casein concentrate using stainless steel membrane. *International Dairy Journal, 80*, 26–34. https://doi.org/10.1016/J.IDAIRYJ.2018.01.002

Zhao, W., Tang, Y., Lu, L., Chen, X., & Li, C. (2014). Review: Pulsed electric fields processing of protein-based foods. *Food and Bioprocess Technology, 7*(1), 114–125. https://doi.org/10.1007/S11947-012-1040-1