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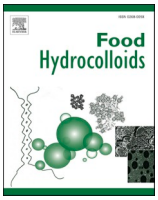

Pressure, shear, thermal, and interaction effects on quality attributes of pea–dairy protein colloidal dispersions

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ABSTRACT

This study evaluated the feasibility of producing stable protein colloidal dispersion by blending dairy and pea proteins using ultra shear technology (UST). The influence of different UST process parameters (shear, temperature, pressure, and interactions) on the quality attributes of milk and pea protein suspensions of different protein proportions viz., milk:pea 1:0.5, 1:1, and 1:3 protein ratios was evaluated. UST treatment was performed at 400 MPa pressure and process temperatures of 40 and 70 °C. High pressure processing (HPP) at 400 MPa at 25 ± 2 °C and thermal treatment at 72 °C were conducted to study pressure-only and temperature-only effects. UST treatment reduced particle size at lower protein concentrations, however, aggregation of particles was observed above certain concentrations. For example, for the UST treatment at 40 °C, the mean particle size of milk:pea 1:0.5 and 1:3 protein suspensions were 2.48 and 23.06 μm, respectively. HPP and thermal treatments did not alter the particle size of the samples. UST treatment produced stable colloidal dispersions, with no sedimentation up to 15 days storage at 4 °C. UST treatment created homogenous stable dairy–pea protein blends, emulsions, or gel structures depending on treatment intensity and protein concentration.

1. Introduction

Foods of today are intended not only to satisfy hunger but also to supplement nutrients required for health benefits. Increasing consumer awareness of health-promoting foods and demand for nutritional protein-based diets has led the food industry to develop cost effective and sustainable protein foods. In the past decade, newer products, such as plant protein-based drinks, that enable balanced nutrition have found their way into the diet. Several dairy milk-substitute products based on oat, almond, soy, coconut, pea, and others are available in the market (Sethi, Tyagi, & Anurag, 2016; McClements, Newman, & McClements, 2019). Plant-based drink sales in the U.S. increased by 61% between 2012 and 2018 whereas sales of dairy milk have declined by 15% since 2012 (Devenyns, 2019). However, plant-based drinks have limited acceptance due to the beany, nutty flavors and bitter taste in some foods and their inability to create flavors similar to dairy milk, especially when used as coffee, tea, or cooking ingredients (McClements et al., 2019). 'Flexitarians' who often move between plant- and dairy-based foods prefer products closer to a natural dairy taste. Incidentally, about 33% of plant-based drink consumers in the U.S. move back to dairy milk due to compromise in taste (Ku, 2020). Further, while plant sources can provide good quantity protein, the functionality and quality of dairy-based milk protein are unmatched. Therefore, blending the plant and dairy protein to create a 'hybrid product' may lead to development of desirable textural and functional liquid beverages that may attract consumers.

Pea protein has received increasing attention as a plant protein with high nutrition content, low price, and sustainability (Lan, Xu, Ohm, Chen, & Rao, 2019). However, pea proteins possess low solubility and settle down as sediments during processing and storage, leading to non-uniformity in protein distribution in protein-enriched beverages and milk substitutes. The poor functional performance of pea proteins, such as their emulsion stability and gelation properties that lead to sedimentation of pea proteins, pose challenges to its inclusion in liquid foods (Choi & Han, 2001; Nosworthy, Tulbek, & House, 2017). Nichols and Cheryan (1982) observed that the functionality of relatively poor functional proteins can be improved by blending with dairy proteins.

The high pressure-based novel ultra shear technology (UST) presents

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a promising way to blend proteins from plant and dairy sources through combined application of high pressure, shear, and thermal exposure. In UST processing, the liquid blend is pressurized up to 400 MPa and passed through a tiny nozzle that exerts enormous shear and concomitant momentary temperature rise on the product. UST is also referred as high-pressure homogenization (HPH) (Maresca, Donsì, & Ferrari, 2011; Ruiz-Espinosa et al., 2012).

Researchers have been investigating the cumulative effect of HPH (cumulative effect of pressure-heat and shear) on various liquid foods including raw bovine milk (Pereda, Ferragut, Quevedo, Guamis, & Trujillo, 2007; Thiebaud, Dumay, Picart, Guiraud, & Cheftel, 2003) and cream (Rodarte, Zamora, Trujillo, & Juan, 2018). The treatment reported to be effective in inactivation of bacteria, and improve the safety and shelf life. Martínez-Monteagudo et al. (2017) studied the influence of pressure-thermal-and shear on the stability, rheological changes, and particle size of a model soy-milk beverage. Authors observed that the high pressure induced shear during treatment caused micro and macro structural modifications in the components, thus modifying the particle size and rheological behavior of the beverage. Further, the treatment enabled stability in beverages while minimizing use of stabilizers, thus opening opportunities for clean label foods. In another study, the effectiveness of the technology in inducing structural changes in fat globules and casein micelles in milk and promoting stability by preventing creaming was noted (Janahar, Marciniak, Balasubramaniam, Jimenez-Flores, & Ting, 2021). Limited studies evaluated individual UST process parameters (pressure, shear, and temperature) and their interactions during UST on the quality attributes and flow behavior of plant–dairy protein blend.

Therefore, the aim of the present work is to study the effects of pressure, shear, temperature, and their interactions on the different quality attributes of milk-plant protein blends of different protein proportions. Pea protein was chosen as a model plant protein. Milk:pea suspensions containing different ratios of milk protein to pea protein, namely, 1:0.5, 1:1 and 1:3 were treated by pressure, shear, temperature, and combinations. Both the individual and interaction effects on the blends were characterized using different quality attributes such as particle size, zeta potential, pH, rheological analysis, sedimentation index, and microstructure. The ability of the UST to obtain homogenous blends and modify the consistency of blends to create products for different applications and attain storage stability of products is evaluated. This study provides critical information for formulating novel protein beverages and designing UST equipment to process these beverages.

2. Materials and methods

2.1. Materials

Milk (2% fat, 3.3% protein) was purchased from local supermarket (Kroger Co., OH) and stored at 4 °C until use. Pea protein powder with 80% protein, 8% total carbohydrate and 8% total fat was purchased from Judee's Gluten Free (DeJa' GF Foods, Plain City, OH) and filtered through sieves to obtain particle sizes of 500–600 μm. Bicinchoninic acid (BCA) assay kit with BCA reagent A and B, and bovine serum albumin (BSA) (2 mg/ml in 0.9% aqueous) were purchased from Pierce Biotechnology Inc./Thermo Fisher Scientific (Rockford, IL).

2.1.1. Ultra shear technology laboratory tester

A laboratory scale Ultra Shear Technology™ (UST^{TM}) tester (Pressure BioSciences (PBI), South Easton, MA, USA) described by Janahar et al. (2021) was used for ultra shear treatment experiments. The equipment involves a pressure chamber where the fluid pressure is increased up to 400 MPa. The pressurized fluid is decompressed by passing through a shear valve, which comprises a spherical ceramic ball placed on a circular seat. When the fluid pressure overcomes the force on the ball, the fluid flows through the gap between the ball valve and seat through an outlet tube to be collected. Pressure transducer and thermocouples are fitted at several locations and the data was recorded using a data acquisition system (PBI, South Easton, MA, USA).

2.2. Methods

2.2.1. Preparation of milk–pea protein suspensions

Different amounts of pea protein powder were added to milk (500 mL) to prepare suspensions with milk protein to pea protein ratios of 1:0.5, 1:1, and 1:3. They were stirred at 350 rpm at room temperature for 3 h to fully hydrate the pea proteins.

2.2.2. Ultra shear treatment

To study the influence of interaction of pressure + shear and pressure + shear + temperature, milk-pea suspensions were treated by UST at 400 MPa at temperatures of 40 and 70 °C. Initial temperatures of 15 ± 2 °C and 25 ± 2 °C were used to achieve 40 and 70 °C, respectively, at exit of shear valve. During 40 °C UST process runs (to evaluate pressure + shear effects), a cooling pad was wrapped around the shear valve body to act as heat sink to keep exit fluid temperature <40 °C. Thus, 400 MPa, 40 °C UST treatment is assumed to have minimal thermal effects. Similarly, for 70 °C UST experiments (to evaluate pressure + shear + thermal effects), to minimize the heat loss during the experiments, the shear valve body was heated using an external heater cartridge (maintained at 70 °C). In both 70 and 40 °C UST experiments, the treated samples were collected at the exit of shear valve from the third process run onwards to ensure steady state process temperature.

For a typical process run (or cycle) of UST treatment, ~2.5–3 mL of temperature preconditioned milk:pea suspensions were fed into the pressure chamber of the UST laboratory tester to be compressed up to 400 MPa followed by passage through shear valve and exit through an outlet tube where the samples are collected. Samples collected were immediately placed in ice-water bath and stored at <4 °C until analysis. Flow rates of milk:pea suspensions were 0.0012 kg/s – 0.0013 kg/s throughout study. Several process runs were carried out continuously to collect required sample for analysis.

2.2.3. Control samples

The untreated milk and milk:pea suspensions of three different protein ratios (1:0.5, 1:1, and 1:3) were used as (no-treatment) controls.

Pressure during high (isostatic) pressure (HPP) and high pressure followed by shear (UST) are generated and discharged using two distinct physical principles though the technologies may utilize same magnitude of high pressure. Thus, the UST bench scale equipment cannot be used for producing isostatic pressure. Due to this technical challenge, (isostatic) high pressure experiments were conducted using a different laboratory scale bench (isostatic) high pressure processor (PT1, Avure Technologies, Kent, WA, USA) as described earlier by Dhakal, Giusti, and Balasubramaniam (2016). Pouches containing milk:pea suspensions (~2.5 mL) at an initial temperature of 13 ± 2 °C were processed at 400 MPa pressure for holding time of 0 min (come-up time) at a process temperature of 25 ± 2 °C.

Similarly, to investigate contribution of temperature-only effect, thermal treatment was performed using MicroThermics UHT/HTST Lab-25HV (MicroThermics, Inc., Raleigh, NC) processing system at 72 °C and 15 s and cooling to 5 °C at a flow rate of 0.05 kg/s.

To investigate the influence of conventional homogenization process, two stage homogenization representing normal manufacturing conditions for dairy products were used (Schmidt & Smith, 1988). Milk:pea suspensions were processed at pressure of 2000 psi (13.79 MPa) in first stage and 500 psi (3.45 MPa) in second stage at temperature of 70 °C and flow rate of about 0.014 kg/s in a two-stage homogenizer (Model NS2002H, GEA Niro Soavi, Parma, Italy).

2.3. Characterization of processed milk-pea protein suspensions

The quality attributes of samples were characterized using particle size, zeta potential, pH, dynamic rheological measurements, sedimentation index, protein solubility, and microstructure. These analyses were carried out within 24 h of various treatments.

2.3.1. Particle size and morphology

The morphology of the samples and particle size were characterized using a Laser microscope (Laser Microscope 3D & Profile measurements, Keyence, VK-x200 series, Osaka, Japan). About 5 μL of samples were spread as a thin layer on a glass slide and allowed to air dry at room temperature for 12 h. For each sample, at least three images were captured at 10 × magnification with VK viewer software in 'Easy Mode'. Further analysis of the images was performed to characterize the particles by mean diameter and average height using VK-Analyzer software (Keyence v3.3.0.0). To measure the mean diameter, it was assumed that each particle, represented by dark black area, in the 2D image had a circular shape. The contour of each fat-protein particle was fitted using the 3-point diameter function to obtain the diameter. At least 20 measurements were obtained for each image and averaged to calculate the mean diameter. The height denotes the vertical distance between the flat base of the glass slide, where the samples were spread, to the top edge of the particle. The measurement of average height was performed based on confocal profiling in the laser microscope (Funke et al., 2015). The laser microscope allowed capture of 3D images to analyze the height of particles.

To compare the contribution of particle diameter in milk to the particle size parameters in milk:pea suspensions, the particle size parameters of water:pea suspensions with similar amounts of pea protein as were added in the milk:pea protein suspensions were measured.

2.3.2. Protein solubility

Protein solubility was determined according to the method of Boye et al. (2010) with slight modifications. The samples (5 ml) were taken in 15-ml tubes and centrifuged at 4000 g for 60 min at 20 °C in Sorvall Legend XFR centrifuge (Thermo Scientific, Waltham, USA). Protein in the initial sample and supernatant portion after centrifugation was determined by BCA assay (Smith et al., 1985). The samples were diluted 1:1000 (v/v) with distilled water to get the range of concentrations that fit the standard curve. For the BCA assay, 25 μL of standard or sample and 200 μL of working reagent (50 parts of BCA reagent A and 1 part of BCA reagent B) were added into wells in a micro plate and incubated at 37 °C for 30 min. The absorbance was measured at 562 nm with Fisherbrand accuSkan GO UV/Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Blank values were determined by analyzing distilled water with no protein and the blank absorbance values were subtracted from the sample absorbance values. The sample absorbances were substituted in the equation obtained from standard curve to obtain the protein concentration. Protein solubility is given as the percent ratio of protein in the supernatant to percent ratio of the total protein in the sample before centrifugation.

2.3.3. pH and zeta potential

The pH was measured using a benchtop pH meter (Mettler-Toledo, USA). Zeta potential measurements of all samples diluted with ultrapure water in the ratio of 1:1000 was performed in a zeta potential analyzer (NanoBrook, ZetaPALS, Brookhaven, Holtsville, NY). The electrophoretic mobility of particles was measured using Phase Analysis Light Scattering technique with a detection angle of 15°. Smoluchowski model was used determine zeta potential from mobility data.

2.3.4. Rheological measurements

The viscoelastic characterization of samples was performed in a Discovery HR3 hybrid rheometer (TA instruments, New Castle, DE, USA). A parallel plates geometry (40 mm plate diameter) with a plate gap of 1000 μm was used. The testing was kept at 25 °C using a Peltier system. Strain sweep measurements were carried out between 0.1 and 1000% strain at a frequency of 1 Hz to determine the linear viscoelastic region of the sample. A strain of 1%, within the linear viscoelastic region was selected and a frequency sweep was performed with frequency ranging from 0.1 to 100 rad/s. The elastic or storage modulus (G′), viscous or loss modulus (G″), tangent of phase angle, δ (tan δ = G″/G′) and the complex viscosity (η*) were obtained as a function of the angular frequency (ω). Steady state flow measurements were carried out at increasing shear rates from 0.1 to 100 s^{-1} to measure the shear viscosities of the samples. Measured viscosities indicate the impact of protein concentrations and the different processing conditions on the flow behavior and stability of the samples. Rheological data were obtained directly from the TRIOS software (TA Instruments, New Castle, DE, USA). Measurements were done in triplicates and the average results are shown.

2.3.4.1. Rheological modeling. To characterize the rheological behavior of samples under different processing conditions in the present study, common rheological models such as Newtonian, Power law or Ostwald-De-Waele, Bingham, and Casson (Eqs. (1)–(4)) were used (Holdsworth, 1971; Deboni et al., 2013).

$$\text{Newton model } \tau = \mu\dot{\gamma} \tag{1}$$

where, τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), and μ is the viscosity (Pa.s).

$$\text{Power law model } \tau = k\dot{\gamma}^{n} \tag{2}$$

k is the consistency index (Pa.s^n) and n is the flow behavior index value (dimensionless). When n = 1, n < 1, and n > 1, the fluid is called Newtonian, pseudoplastic, and dilatant, respectively.

$$\text{Casson model } \tau^{0.5} = k_{oc} + k_c\dot{\gamma}^{0.5} \tag{3}$$

Here, k_{oc} is Casson's initial stress (Pa) and k_c is Casson consistency index (Pa.$s^{0.5}$).

$$\text{Bingham model } \tau = \tau_0 + \eta_p\dot{\gamma} \tag{4}$$

Here, τ_0 is initial shear stress (Pa) and η_p is the Bingham's consistency index, also referred as called plastic viscosity (Pa.s).

The best model was selected based on the simplicity and higher correlation coefficient (R) between observed and estimated values of shear stress (Deboni et al., 2013).

$$R = \frac{\sum (x-\bar{x})(y-\bar{y})}{\sqrt{\sum (x-\bar{x})^2 \sum (y-\bar{y})^2}} \tag{5}$$

In addition, the standard error of estimates (SEE) were determined using Eq. (6).

$$SEE = \sqrt{\frac{1}{(n-2)}\left[\sum (y-\bar{y})^2 - \frac{[\sum (x-\bar{x})(y-\bar{y})]^2}{\sum (x-\bar{x})^2}\right]} \tag{6}$$

Where, x is the observed value, y is the estimated value, $\bar{x}$ and $\bar{y}$ are mean of observed and estimated values, respectively and n is the count of the total number of (x,y) pairs.

2.3.5. Sedimentation index

Sedimentation index was determined using the method described by Kubo, Augusto, and Cristianini (2013) with minor modification. Ten mL samples were taken in graduated, sterile, screw-capped polypropylene centrifuge tubes (Fisher Scientific Ltd., cat. no. 05-539-12) and stored at ≤4 °C for 15 days. The volume of the supernatant milk phase and the sediment volume, due to the sedimentation of pea protein solid particles were measured every 24 h from the graduations in the tubes. The sedimentation index (%) was determined using the following equation:

$$\text{Sedimentation index } (\%) = \left(\frac{\text{Sediment volume}}{\text{Total sample volume}}\right) \times 100 \quad (7)$$

2.3.6. Microstructure

For laser microscope analyses, samples (5 μL) spread and air dried on a glass slide were observed with lens of 10 × magnification by 2D and 3D images obtained by a non-contact 3D laser scanning microscope (LSM) (VK-X200 series, Keyence, Osaka, Japan).

For scanning electron microscope (SEM) analysis, Thermo Scientific Quattro Environmental Scanning Electron Microscope (ESEM) was used. The samples were frozen by immersion in liquid nitrogen and freeze-dried (VirTis Benchtop K, model #2KBTES, SP Scientific, PA, USA). Afterwards, all the samples were mounted using carbon tape on aluminum stubs (SPI Supplies), sputtered with gold in Pelco Model 3 sputter coater and analyzed under SEM at a voltage of 5 kV, current of 0.18 nA and pressure of 0.001 Pa at 350 × magnification.

2.4. Statistical analysis

All the processing runs and instrumental analysis were carried out in triplicate, unless mentioned otherwise. The significance of analysis results with respect to different treatments, protein concentrations, and interactions was investigated by general linear model (GLM) univariate ANOVA at a significance level of 0.05 using SPSS (version 27, IBM SPSS Statistics, Armonk, NY). The different dependent variables were analyzed using a 2-way ANOVA, with the factors - protein concentration and treatment. For each treatment, the effect of different protein concentrations and for each protein concentration, the effect of different treatments on analysis results were determined by ANOVA and Tukey Honest Significance Difference (HSD) test was applied to compare means.

3. Results and discussion

3.1. Pressure-thermal history of UST-treated samples

The representative pressure-temperature history of UST treated samples at 40 °C and 70 °C are shown in Fig. 1-A (a,b). During UST treatment, the fluid preconditioned at an initial temperature and pressurized to 400 MPa within a pressure chamber. The fluid temperature transiently increases by heat of compression by 3 °C per 100 MPa (Rasanayagam et al., 2003). Then the fluid exits through the shear valve, where the pressure energy is converted into kinetic energy due to the pressure drop (ΔP = 400 MPa).The kinetic energy is dissipated as temperature increase in the product temperature, help to modify liquid food structure and heat loss to the surrounding.

The theoretical temperature rise by shearing can be estimated by equating the work done on the fluid by pressure to the heat energy ($pdV = mc_p\Delta T$). For water at 25 °C, the temperature rise (ΔT) can be estimated as 26.20 °C per 100 MPa (Janahar et al., 2021). Unlike heat of compression, such temperature increase due to heat of homogenization is irreversible. The magnitude of temperature increase influenced in part influenced by the flow rate, and heat loss to the environment. For example, researchers reported temperature rise between ~15 and 20 °C per 100 MPa (Martínez-Monteagudo et al., 2017; Zamora, Ferragut, Guamis, & Trujillo, 2012; Pereda et al., 2007) when using pilot scale systems typically operated at higher flow rates (0.026–0.034 kg/s). Higher flow rates help to stabilize the temperature and minimize heat loss to the environment. Additionally, this pilot scale equipment utilized twin pressure chambers which help to smoothen and maintain steady flow. The bench scale UST equipment used in the study (0.0012–0.0013 kg/s) on the other hand utilized single pressure chamber, shear valve and external heaters to produce desired high pressure and shear effects. Though this helped to conduct experiments under controlled pressure, shear and thermal conditions, treatment produced intermittent flow. This in turn resulted in contributed to larger heat loss to the

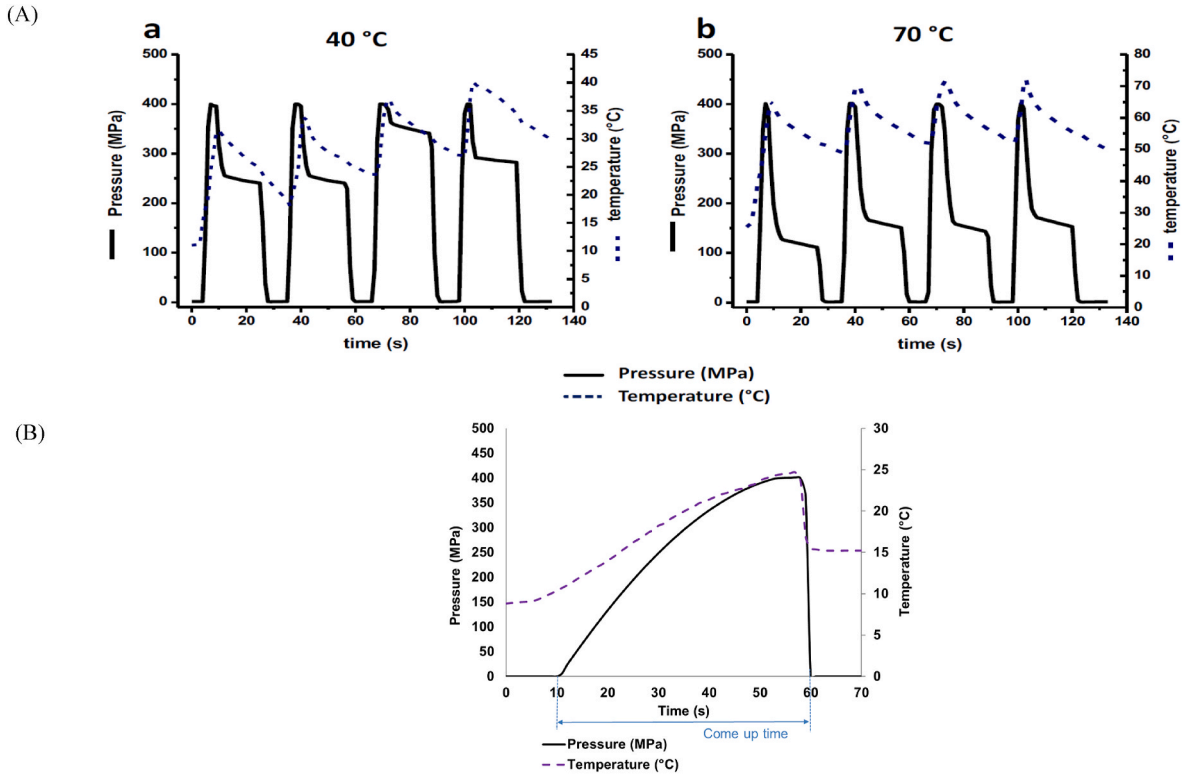

Fig. 1. (A) Representative pressure – temperature history during ultra shear treatment of liquids at two different temperatures [a) 40 °C and b) 70 °C] (B) Representative pressure – temperature history during HPP treatment of liquids.

environment when the fluid exit the shear valve. As a result, the heat of homogenization of 11.57 ± 0.08 °C and 6.82 ± 0.47 °C per 100 MPa were observed for 70 and 40 °C UST runs.

Fig. 1-B represents the pressure-thermal history during high pressure processing of the fluid at 400 MPa and 25 °C process temperature. It is worth noting that in HPP treatment, the pressure come-up time (≈48 s) was longer than UST (≈1.5 s). Similarly, thermal only experiments had a come-up time about 15 s to reach 72 °C with 15 s hold time. Due to technical challenges associated with equipment, the study did not consider the influence of different process come-up times on various characteristics of treated milk-pea suspensions.

3.2. Particle size characterization and morphology

The impact of the pressure, shear, temperature, and interactions on the particle size parameters, namely, mean diameter and average height, are shown in Table 1. In untreated milk:pea samples at protein ratios from 1:0.5 to 1:3, the mean diameter of particles varied between 32 and 38 μm and the average height varied between 58 and 87 μm with no significant differences between protein ratios (see Table A1). The average diameter values were close to the average diameter of 39.50 μm for commercial pea protein isolate, reported by Osen, Toelstede, Wild, Eisner, and Schweiggert-Weisz (2014).

After 40 °C UST treatment, for milk:pea samples at 1:0.5 and 1:1 protein ratio, the mean diameter and height reduced significantly ($P <$ 0.05). For example, the maximum diameter was less than 3 μm and average height was less than 10 μm. For milk:pea samples at 1:3 protein ratio the mean diameter and average height were ~23 μm and 56 μm respectively, significantly higher than the low protein concentration samples (milk:pea 1:0.5 and 1:1) and not significantly different from untreated samples. Similarly, after 70 °C UST treatment of milk:pea 1:0.5 and 1:1 protein samples, the mean diameter reduced significantly ($P < 0.05$) up to 4 and 5 μm, respectively, and average height reduced significantly ($P < 0.05$) up to 13 and 20 μm,respectively. For milk:pea 1:3 protein samples, the mean diameter and average height were 44 μm and 30 μm, respectively, which were significantly higher than the low protein concentration samples and not significantly different from untreated samples. The mean particle diameter of milk (2% fat) with no inclusion of pea protein was about 1 μm, which was reduced upto 0.24 μm and 0.29 μm after 40 and 70 °C UST treatments respectively. This indicated that the particle size of milk:pea dispersions was mainly contributed by pea protein particles.

Table 1
Particle size parameters of samples treated by different processing methods.

S. No.	Treatment	Milk:Pea protein ratio	Mean diameter (μm)	Average height (μm)
1	Untreated	1:0.5	31.90^{aB} ± 2.46	87.36^{aAB} ± 25.64
		1:1	35.58^{aB} ± 1.37	78.18^{aC} ± 16.41
		1:3	37.89^{aB} ± 7.31	58.34^{aA} ± 7.72
2	UST-400 MPa-40 °C	1:0.5	2.48^{aD} ± 0.05	8.54^{aD} ± 0.68
		1:1	2.56^{aA} ± 0.68	10.40^{aA} ± 3.92
		1:3	23.06^{bAB} ± 8.44	56.58^{aA} ± 48.95
3	UST-400 MPa-70 °C	1:0.5	3.83^{aD} ± 0.43	13.43^{aD} ± 3.13
		1:1	4.63^{aA} ± 1.41	20.15^{abAB} ± 6.91
		1:3	44.10^{bB} ± 13.08	29.98^{bA} ± 5.79
4	HPP-400MPa-25 °C	1:0.5	29.31^{aB} ± 5.56	68.64^{aBC} ± 1.69
		1:1	30.05^{aB} ± 2.51	89.59^{aC} ± 16.09
		1:3	27.41^{aAB} ± 4.01	67.48^{aA} ± 11.47
5	Thermal treatment-72 °C	1:0.5	44.42^{aA} ± 2.86	50.55^{aC} ± 4.60
		1:1	36.38^{aB} ± 4.57	80.74^{aC} ± 15.15
		1:3	42.77^{aB} ± 9.88	62.70^{aA} ± 21.30
6	Homogenization-2000psi-500psi-70 °C	1:0.5	10.79^{aC} ± 2.54	100.21^{aA} ± 4.01
		1:1	11.15^{aA} ± 5.91	66.51^{abBC} ± 31.16
		1:3	24.89^{bA} ± 4.35	35.11^{bA} ± 7.44

[1]Values are expressed as Mean ± Standard Deviation.
[2]For each treatment, means in same column without common lower-case superscripts are significantly different ($P \leq 0.05$); For each protein ratio, means in same column without common upper-case superscripts are significantly different ($P \leq 0.05$).

It was also noted that for milk:pea 1:3 protein samples, 70 °C UST treatment caused significantly higher ($P < 0.05$) particle diameter than the 40 °C UST treatment. This increased diameter indicated the effect of temperature in UST on the particle size modification in the sample. Protein-stabilized emulsions could aggregate at high temperature as the protein–protein associations bind the particles together in a network (Sliwinski, Roubos, Zoet, van Boekel, & Wouters, 2003). Further, the pea particles have globular proteins adsorbed to the surfaces, which may naturally have a relatively high surface hydrophobicity. These proteins may become more hydrophobic due to surface disturbances or thermal denaturation (McClements et al., 2019). The shear led surface changes to the proteins and the pressure-shear-temperature led protein denaturation might have resulted in increased particle size in high protein concentration samples treated by 70 °C UST. Our results demonstrated the feasibility of formulating liquid foods with varying consistency and food structure by suitably varying protein concentration and UST-treatment parameters.

Unlike UST, pressure-only treatment did not cause significant reduction in mean particle diameter and average height for all protein ratios. Several researchers have reported that HPP at pressures up to 600 MPa and holding times up to 3 min does not cause significant reduction in the average fat globule size in milk (Huppertz, Fox, & Kelly, 2003; Stratakos et al., 2019; Ye, Anema, & Singh, 2004) and cream (Dumay, Lambert, Funtenberger, & Cheftel, 1996) as compared to untreated samples. The particle size of 5.0% whey protein isolate mixture did not significantly change after HPP treatment at 450 MPa for 3.5 min at 5–10 °C (Zhang et al., 2020). Likewise, the thermal treatment did not create significant change in the particle size parameters of suspensions, making the temperature contribution in particle size change negligible. Dhakal et al. (2016) reported that the average particle diameters of almond drink treated at 72 °C (for 300 and 600 s) did not alter significantly as compared to raw almond drink.

After homogenization treatment of milk:pea 1:0.5 and 1:1 protein samples, the mean diameter reduced significantly ($P < 0.05$) up to 11

μm. The diameters were lesser than untreated samples but greater than UST-treated samples and these samples eventually exhibited sedimentation of pea protein particles during storage (discussed in section 3.6). For milk:pea 1:3 protein samples, the mean diameter was significantly higher at 25 μm, but not significantly different from untreated samples. These samples had viscosities less than UST-treated milk:pea 1:3 samples (discussed in section 3.5.3). It is worth noting that study did not consider how various treatments (pressure, temperature, shear) influence different types of pea and dairy proteins in various confirmations and should be a topic of future research.

3.3. Protein solubility

The protein solubility provides useful information on effective utilization of the products in various food applications (Boye et al., 2010). The solubilities of milk:pea samples of different protein ratios treated by different treatments are shown in Table 2. Analysis of variance indicated that the influence of different treatments was significant, while protein concentration and interaction of protein concentration and treatment were not significant. The recorded solubilities agreed with previous reports by Shand, Ya, Pietrasik, and Wanasundara (2007) and Osen et al. (2014) for commercial pea protein isolates. However, the relatively lower solubility could be because of initial denaturation in the pea protein during the manufacturing process and is in agreement with the normal properties of pea protein in general. The untreated milk:pea samples of 1:0.5, 1:1, and 1:3 protein ratios were 28.23, 22.67, and 17.27%, respectively. Protein solubilities of samples treated by the HPP, thermal, and homogenization treatments were not significantly different from untreated samples.

The solubilities of 70 °C UST-treated samples varied from 31.28 to 34.47% and were significantly higher than untreated samples, although not significantly different from 40 °C UST-treated samples. Thus, effects of pressure-associated shear and temperature during UST on solubility is evident. The solubilities could be increased because the shear treatment caused size reduction of large protein particles and rearrangements in the protein aggregates, thus enabling protein–solvent interaction, and making the proteins accessible for reaction similarly as is the case with the casein micelles which are in colloidal suspension (Chen, Xu, & Zhou, 2016; Moll, Salminen, Schmitt, & Weiss, 2021).

3.4. pH and zeta potential

The pH of untreated milk:pea samples of different protein ratios varied from 6.70 to 6.73 with no significant difference ($P < 0.05$) between protein ratios (Table A2). The pH of milk:pea samples treated using different processes were not significantly different from untreated samples. The observations were consistent with earlier research (Pereda et al., 2007; Janahar et al., 2021). Above the isoelectric point, the pea globulins are not dissociated completely and so the surface-active material of the protein is less available for adsorption at the interface of fat-protein (Gharsallaoui, Cases, Chambin, & Saurel, 2009). In addition, the structure of casein micelles were changed by treatments only at pH lower than natural milk pH (Huppertz, Fox, & Kelly, 2018). Therefore, the pH variation and its effect in particle conformational changes and particle interactions can be negligible.

The zeta potential indicates magnitude of charge on a colloidal particle. The terms 'increase or decrease' are not used algebraically and represent the increase or decrease of numerical value of zeta potential. The different treatments, protein ratio, and interactions had significant effect ($P < 0.05$) on the zeta potential of samples (see Table A2). The zeta potential of untreated samples of milk:pea suspensions from 1:0.5 to 1:3 ranged from −43.83 to −47.87 mV. The zeta potential of untreated water:pea dispersions with similar pea protein concentrations ranged from −36.78 to −41.37 mV and these were significantly different ($P < 0.05$) from untreated milk:pea suspensions. The isoelectric point of pea proteins and casein protein in milk are 4.5 and 4.6, respectively (Tomé, Pires, Batista, Sousa, & Raymundo, 2014). The pH of all samples were apparently above the isoelectric point and this pH was responsible for the negative values of zeta potential. In untreated, HPP-, and thermal-treated samples the zeta potential of milk:pea 1:3 samples were significantly higher ($P < 0.05$) than the other samples. This higher zeta potential might be due to increased protein concentration in 1:3 samples. Further, the higher zeta potential of thermally treated samples as compared with other samples might be due to rearrangement of the protein particles or network caused by heat exposure (Tholstrup Sejersen et al., 2007).

In 40 and 70 °C UST treated samples, the zeta potential values decreased respectively from −46.23 to −29.16 mV and −46.70 to −34.32 mV with increasing protein ratios from 1:0.5 to 1:3 in milk:pea blends. A significant decrease ($P < 0.05$) in zeta potential with increasing protein concentration under UST treatment was noted. This decrease might be because the UST treatment could make the charged

Table 2
Solubility of samples treated by different processing methods.

S. No.	Treatment	Milk:Pea protein ratio	Soluble protein (mg/mL)	Insoluble protein (mg/mL)	Total protein (mg/mL)	Protein solubility (%)
1	Untreated	1:0.5	13.27 ± 3.75	33.67 ± 8.75	46.94 ± 12.50	28.23^{aAB} ± 0.34
		1:1	15.38 ± 2.50	53.12 ± 15.63	68.50 ± 18.13	22.67^{bAB} ± 1.69
		1:3	22.04 ± 4.37	108.34 ± 43.13	130.38 ± 47.50	17.27^{cAB} ± 2.15
2	UST-400 MPa-40 °C	1:0.5	15.82 ± 1.25	34.25 ± 16.88	50.06 ± 18.13	32.75^{aB} ± 6.84
		1:1	14.93 ± 3.13	58.88 ± 8.75	73.81 ± 11.88	20.17^{bAB} ± 0.70
		1:3	40.11 ± 3.75	101.20 ± 1.88	141.31 ± 5.63	28.36^{abAB} ± 1.08
3	UST-400 MPa-70 °C	1:0.5	13.15 ± 1.25	29.10 ± 6.25	42.25 ± 7.50	31.28^{aB} ± 1.85
		1:1	24.02 ± 0.63	49.49 ± 0.01	73.50 ± 0.63	32.68^{abB} ± 0.40
		1:3	42.36 ± 1.25	80.52 ± 2.50	122.88 ± 3.75	34.47^{cB} ± 0.02
4	HPP-400 MPa-25 °C	1:0.5	13.29 ± 1.88	29.58 ± 12.50	42.88 ± 14.38	31.72^{aB} ± 4.55
		1:1	14.50 ± 0.63	56.19 ± 27.50	70.69 ± 28.13	21.56^{aAB} ± 5.66
		1:3	37.64 ± 10.63	95.86 ± 14.38	133.50 ± 25.00	28.03^{aAB} ± 1.93
5	Thermal treatment-72°C-15s	1:0.5	12.56 ± 0.63	40.00 ± 21.25	52.56 ± 21.88	25.24^{aAB} ± 6.88
		1:1	20.38 ± 11.88	53.12 ± 1.88	73.50 ± 13.75	27.04^{aAB} ± 7.92
		1:3	19.37 ± 3.13	103.60 ± 99.37	129.13 ± 102.50	24.89^{aAB} ± 14.40
6	Homogenization-2000psi-500psi-70 °C	1:0.5	7.58 ± 1.25	36.23 ± 0.63	43.81 ± 1.88	17.27^{aA} ± 1.50
		1:1	15.46 ± 9.38	67.74 ± 0.01	83.19 ± 9.37	18.23^{aA} ± 6.54
		1:3	20.27 ± 1.25	110.10 ± 56.25	130.38 ± 57.50	16.51^{aA} ± 4.69

[1]Values are expressed as Mean ± Standard Deviation.
[2]For each treatment, means in same column without common lower-case superscripts are significantly different ($P \leq 0.05$); For each protein ratio, means in same column without common upper-case superscripts are significantly different ($P \leq 0.05$).

amino acid residues move from the surface of the protein to its interior and/or create protein–protein linkage, thus masking negative charges (Relkin & Shukat, 2012). Similar behavior of decrease of zeta potential of samples due to UST treatment was reported by Janahar et al. (2021) in raw milk and this behavior was attributed to the surface modifications in proteins caused by pressure-associated shear. The values obtained for high protein concentration (milk:pea 1:3) samples were closer to −24 mV and −30 mV observed for hazelnut drink (Bernat, Cháfer, Rodríguez-García, Chiralt, & González-Martínez, 2015) and almond drink (Bonsegna et al., 2010; Gallier, Gordon, & Singh, 2012), respectively. At milk:pea protein ratio of 1:3, the zeta potential after 70 °C UST was higher than 40 °C UST, which might be attributed to relatively higher protein conformational changes possibly affected by UST temperature and increased particle size in 70 °C UST treatment. Relkin and Shukat (2012) reported increases in surface charge characteristics in parallel with increases in the particle sizes in protein systems.

The zeta potential of homogenized samples varied from −44.41 to −37.66 mV with decreasing value with increasing protein concentration. The decreasing trend was similar to UST treatments and might be attributed to similar surface modifications in proteins caused by shear and cavitation generated by homogenization (Meena, Singh, Borad, & Panjagari, 2016). Thus, the knowledge on zeta potential values of different milk:pea protein suspensions demonstrate the influence of composition, surface modifications of particles, protein conformational changes, particle size changes, and rearrangement of particle networks when treated by different processing technologies.

3.5. Rheological measurements

3.5.1. Strain sweep

The storage modulus (G′) as a function of strain for samples with different treatments is shown in Fig. 2. For untreated, HPP, thermal, and homogenization treatments, all the milk:pea samples from 1:0.5 to 1:3 protein ratios showed a linear relationship with strain. For UST treatments, milk:pea samples with 1:0.5 and 1:1 protein ratios showed linear relationship with strain; however, milk:pea 1:3 samples showed a linear viscoelastic region at the strain <8% followed by a nonlinear region (Fig. 2).

The UST treatments of milk:pea 1:3 samples resulted in higher values of G′ when compared with untreated, HPP-, thermal-, and homogenization-treated samples of all protein ratios. These higher values of G′ evidenced that UST promoted the formation of protein networks at high protein concentration (Nicolai, 2019). Furthermore, UST treatments of milk:pea 1:3 samples at 70 °C showed the highest gel strength, which could be attributed to the presence of more networks originating from partially unfolding of proteins and their subsequent aggregation induced by thermal effects during UST. Further, for both 40 and 70 °C UST treatments, the transitions from the linear to non-linear region for milk:pea 1:3 protein ratio samples were around 8% strain, which is the yielding point above which the structure of the sample is disturbed. This critical strain might be due to the breakdown of the secondary interaction between particles, thus affecting the sample network (Hesarinejad, Koocheki, & Razavi, 2014).

3.5.2. Frequency sweep

Based on the information obtained from strain sweep analysis, the limits of the viscoelastic region were defined and 1% strain was used for frequency sweep measurements. Fig. 3a, Fig.A1 and Fig. 3b shows the storage modulus (G′), loss modulus (G″), and tangent of phase angle (tan δ) respectively as a function of angular frequency.

The change of G′ and G″ with respect to frequency would unravel the difference between solution or gel-like structure, and thus can be used to characterize dispersions such as emulsions, gels, foam, and colloidal dispersions (Xiu, Zhou, Zhu, Wang, & Zhang, 2011). In the present study, the magnitudes of both G′ and G″ increased with frequency for all samples and treatments. For the untreated, HPP, and thermal treatments of all samples and homogenization treatments of 1:0.5 and 1:1 milk:pea samples, the values of G′ and G″ were nearly constant at low frequencies and values increased at higher frequencies. Homogenization of milk:pea 1:3 samples resulted in slightly higher values of G′ and G″ indicating an increase in the viscoelasticity of the sample.

UST treatment of samples processed at 40 and 70 °C resulted in higher G′ and G″ values with increased protein ratios from 1:0.5 to 1:3. Notably, in both 40 and 70 °C UST treatments of milk:pea 1:3 samples, the values of G′ and G″ were clearly higher than the rest of the samples. The G′ of all samples (except UST treated milk:pea 1:3 samples) showed a strong dependency of frequency (Fig. 3a). In contrast, the G′ of UST treated milk:pea 1:3 samples were almost constant with increasing frequency at frequency less than 30 rad/s, indicating the formed structures have relatively rigid structure which allows them relax quickly. This is probably due to the presence of more cross-links in the system (Fig. 3a). The values of 70 °C UST were higher than 40 °C UST, which might emphasize the role of temperature on the UST treatment for desirable texture formation in the products. Thus, UST could increase the gel strength based on protein concentration and process temperature used. Shand et al. (2007) studied the thermal properties of pea protein slurry (10% protein w/w) using differential scanning calorimetry (10 °C/min heating rate) and reported two major endothermic peaks at 67.1 ± 1.8 and 85.1 ± 0.4 °C. Sun and Arntfield (2010) determined the denaturation temperature of commercial pea protein isolate (10.5% w/v) as 72.9 °C. Mession, Sok, Assifaoui, and Saurel (2013) reported that the thermal denaturation of low-denatured pea proteins begins at

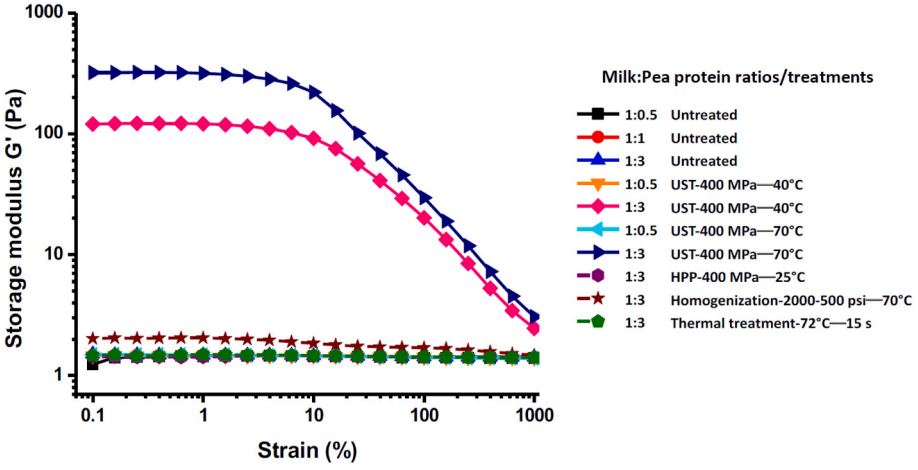

Fig. 2. Strain sweep dependency of storage modulus (G′) of milk:pea protein samples. Frequency = 1 Hz.

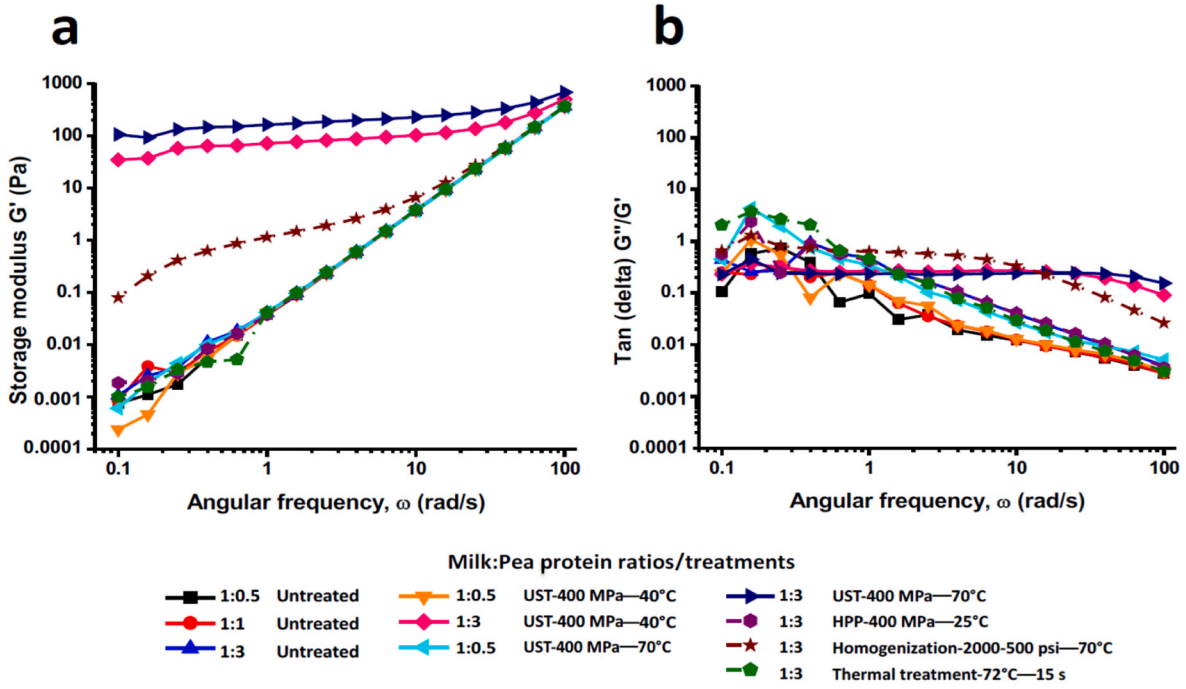

Fig. 3. Frequency sweep analysis of milk:pea protein samples a) Storage modulus, G′, b) Tan δ

temperatures slightly below 70 °C. In addition, the initial denaturation temperatures of whey proteins in milk are 62, 64, and 72 °C for α-lactalbumin, bovine serum albumin, and immunoglobulin G, respectively (Lee, 1992).

Pea proteins may include different types of proteins (such as legumin, vicilin) in various conformations. Our future research will address various treatment impact on some of these proteins. Additional studies on the impact of UST treatment on protein and non-proteinaceous content (such as carbohydrates, which constitutes 20% of pea protein) merits further investigation.

It is interesting to note that, in water and pea suspensions where milk was replaced by water, the G′, G″, and viscosities were much lesser than the milk:pea suspensions with similar protein contribution by pea under UST treatments (data not shown). Thus, the role of milk proteins and fat in the rheological characteristics of milk:pea suspensions is indispensable. Further, values of G′ were higher than G″, which indicated that milk:pea 1:3 samples treated by UST 40 and 70 °C have dominant elastic rather than viscous properties. Therefore, the product can be classified as a weak gel (Martínez-Ruvalcaba, Chornet, & Rodrigue, 2007).

Fig. 3b presents the change of tangent of phase angle (tan δ) with respect to angular frequency, which is used to determine the structural stability of the samples. When tan δ is higher, the proportion of dissipated energy due to a dynamic oscillation at known frequency and low shear amplitude is high (Xiu et al., 2011). For all the samples and treatments, the tan δ were less than unity, which indicated predominant elastic behavior. Further, tan δ decreased with increasing frequency, indicating the rise of elastic behavior at higher frequencies (Xiu et al., 2011). In milk:pea 1:3 protein samples treated by UST 40 and 70 °C, tan δ almost kept constant for a longer range of frequency. This result indicated the higher strength of gel structure of samples treated by UST. The clear tendency of UST to form macromolecular networks and complex structures as compared to other treatments is demonstrated in the measurements. The results also corroborate the differences in particle sizes created by UST treatment as discussed in section 3.2.

3.5.3. Viscosity

In the flow sweep measurements at shear rates of 0.1–100 s^{-1}, the viscosities showed varying behavior with different samples and treatments (Fig. 4). For milk:pea protein at 1:0.5 and 1:1 ratios, untreated, HPP, thermal, and homogenization treatments showed nearly constant viscosities with increasing shear rate, i.e. Newtonian behavior. For 1:3 milk:pea samples, the untreated, HPP, and thermal treatment produced near constant viscosities at rates up to ~5 s^{-1} and increasing viscosities at shear rates over ~5 s^{-1}, although less pronounced, indicating a slight dilatant or shear thickening behavior.

For milk:pea 1:3 samples treated by 40 and 70 °C UST treatments, the viscosities were higher and exhibited shear-thinning behavior (Fig. 4). The pressure-associated shear and temperature in UST promote complex molecular interactions of proteins and fats to create a gel network, thus increasing the viscosity. The viscosities of high protein solutions (≥10% w/v) are contributed predominantly by short-range (a few Å) non-covalent interactions (including van der Waals attractions, dipole−dipole interaction, hydrophobic interactions, and hydrogen bonding), and to a lesser extent by long range (>5 nm) electrostatic interactions. These interactions could be weakened by increasing inter-particular distance during the application of shear deformations during measurements (Chen et al., 2021). Moreover, when the shear rates are lower, the hydrodynamic forces are insufficient to break the linkages between particles. Thus, the viscosity is unchanged. Conversely, at higher shear rates, the hydrodynamic forces are enough to disrupt the linkages. Further, the forces cause molecular realignment in the particles. Thus, the viscosity is reduced (McClements, 2004).

The viscosity of treated samples depended on the protein concentration, applied pressure, and the shear intensity. For instance, the viscosity of low protein concentration samples (milk:pea 1:0.5, 1:1) was less than high protein concentration (milk:pea 1:3) samples. Additionally, the viscosity of homogenization-treated milk:pea 1:3 samples was less than UST-treated milk:pea 1:3 samples. We have also conducted separate UST treatments using several different plant protein sources like soy, mung bean, chia seed, chick pea etc. and found similar observations (data not shown).The plant–dairy protein blend samples of varying viscosities that were treated by pressure-associated shear, based on protein concentration, pressure, and shear intensity resembled products such as liquid beverages, smoothies, protein shakes, cream-type products, sauce-type products, gel-type products, jelly-type products, spread-type products, and egg substitute-type products.

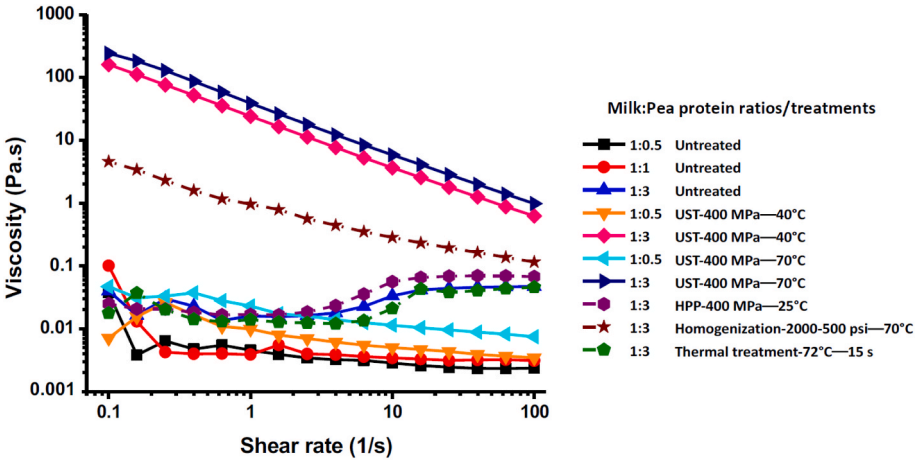

Fig. 4. Viscosity as a function of the shear rate for different samples.

3.5.4. Rheological modeling

The rheological model parameters of milk:pea samples processed by different treatments are shown in Table 3. In Newton's model, the viscosity (μ) for different samples varied from 0.0023 to 0.697 Pa s. The milk:pea protein 1:3 samples treated by 40 and 70 °C UST resulted in higher viscosities of 0.448 and 0.697 Pa s and relatively lesser R values as compared to other samples.

The untreated, HPP-, and thermal-treated milk:pea 1:3 samples showed shear-thickening effects ($n > 1$). In these suspensions, the particles might not be originally aggregated under quiescent conditions owing to a sufficiently high energy barrier between particles. However, during increased shear stress, due to the force of impact and the increased frequency of collisions between the particles, the particles gain sufficient energy to jump over the energy barrier and aggregate (McClements, 2004). This leads to shear thickening behavior.

On the other hand, the suspensions treated by UST were pseudoplastic ($n < 1$), which could be attributed to disruption of linkages in the gel network with increasing shear rate. It should be noted that the Ostwald-De-Waele's consistency index of milk:pea 1:3 samples reached higher values of 24.3 and 39.2 $Pa.s^n$, with n values of 0.19 and 0.19, due to 40 and 70 °C UST treatments, respectively. The thermal effect during UST treatment of these samples on the consistency of blends is thus evident.

Milk:pea 1:3 protein samples showed higher Casson consistency index of 0.390 and 0.482 Pa s after 40 and 70 °C UST treatments, respectively (Table 3). Further, the milk:pea 1:3 protein samples showed higher Bingham's consistency index of 0.45 and 0.70 Pa s after 40 and 70 °C UST treatments, respectively.

3.6. Sedimentation index

Fig. 5 shows the sedimentation index for the untreated samples and samples processed by different treatments under refrigerated storage for 15 days. Samples other than UST treated (including untreated, HPP, and thermal treated) showed sedimentation within the first 24 h of storage under refrigeration whereas UST-treated samples showed no sedimentation throughout storage period. With increasing milk:pea protein ratios from 1:0.5 to 1:3, the sedimentation index of untreated, HPP, and thermal treated samples varied from 11.67 to 58.33%, 18.33 to 75.00%, and 11.67 to 66.67% respectively. The sedimentation index of untreated water:pea suspensions with increasing pea protein concentrations increased from 21.67% to 76.67%. The higher values as compared to corresponding untreated milk:pea suspensions shows the role of milk in stabilizing the pea protein. In milk:pea suspensions sedimentation is contributed by the larger particle size and inability of the milk matrix to keep all the pea particles in suspension. The amount of sedimentation depended on the amount of pea protein added. It was noted that the HPP treatment resulted in significantly different sedimentation index values than untreated samples for milk:pea 1:1 and 1:3 samples. This difference might indicate the effect of HPP to alter the conformation of fat and protein macromolecules and induce aggregates in milk:pea suspensions. Dickinson and James (1998) indicated that HPP could induce significant levels of flocculation in model oil-in-water emulsions stabilized by β-lactoglobulin and the level of pressure-induced flocculation could be controlled by changing the intensity of the HPP. Janahar et al. (2021) reported that HPP of raw milk at 400 MPa for 0 min at 40.66 ± 0.82 °C showed excessive creaming compared to untreated milk due to pressure-induced formation of larger milk fat clusters.

Conventional homogenization of milk:pea 1:05 and 1:1 samples prevented sedimentation only up to 2 days after which the sedimentation index increased up to 11.67 and 18.33%, respectively, indicating that the particle size reduction by homogenization is insufficient to create a stable product.

The UST treatment at 40 and 70 °C prevented sedimentation in all milk:pea samples with 0% sedimentation index up to 15 days. In milk:pea protein 1:0.5 and 1:1 samples, this prevention could be due to reduced fat–protein particle size caused by the pressure-associated shear in UST. In milk:pea 1:3 samples, the stability could be attributed to the increased viscosity of the blend caused by pressure-associated shear. According to Stokes law, the particle sedimentation velocity is directly proportional to its diameter, the acceleration imposed, and the difference of density between the particle and dispersant medium and is inversely proportional to the dispersed medium viscosity. The presence of sedimentation in untreated samples is shown in Fig. 6a and absence of sedimentation in UST-treated (70 °C) samples is shown in Fig. 6b.

To confirm the particle interactions and blending ability of UST treatment, the pH of the milk:pea samples were adjusted to 4.6 using diluted HCl and centrifuged at 4000×*g* for 30 min at 20 °C. The untreated samples showed separation of casein protein and pea protein (Fig. 6c), while the UST-treated samples showed thorough blending of casein and pea protein particles (Fig. 6d).

3.7. Microstructure

3.7.1. Laser scanning microscope (LSM) images of untreated proteins

First, efforts were made to understand the contribution of untreated milk fat and milk protein on particle size through comparison with pea protein by itself suspended in water. The milk fat–protein region is indicated by light green color in 3D image (Fig. A2 a & b). In the figure, circular dark structures represent pea protein and intermittent tiny dark spots represent milk fat or protein. In water–pea suspensions (Fig. A2c), the tiny dark spots in the white region are not observed and this region is

Table 3
Rheological model parameters of samples treated by different processing methods.

S.No.	Treatment	Milk:Pea protein ratio	Newton model parameter			Power law model parameters				Casson model parameters				Bingham model parameters			
			μ (Pa.s)	R	SEE	n	k (Pa.s)	R	SEE	k_{oc} (Pa)	k_c (Pa.s)	R	SEE	η_p (Pa.s)	τ_0 (Pa)	R	SEE
1	Untreated	1:0.5	0.0023	1.00	0.002	0.8319	0.0044	1.00	0.08	0.0176	0.0464	1.00	0.005	0.0023	0.0024	1.00	0.002
		1:1	0.0031	1.00	0.002	0.7678	0.007	0.99	0.11	0.0244	0.0528	1.00	0.01	0.0031	0.003	1.00	0.002
		1:3	0.0475	1.00	0.05	1.0984	0.0247	0.99	0.18	−0.0882	0.2242	1.00	0.06	0.0475	−0.059	1.00	0.05
2	UST-400MPa-40 °C	1:0.5	0.0035	1.00	0.01	0.7663	0.0094	0.98	0.13	0.0405	0.0559	1.00	0.01	0.0035	0.0091	1.00	0.01
		1:1	0.0116	0.99	0.004	0.7606	0.0337	1.00	0.01	0.0809	0.1034	1.00	0.03	0.0116	0.0404	0.99	0.004
		1:3	0.4481	0.90	5.64	0.1904	24.322	1.00	0.01	4.4617	0.3903	0.97	0.31	0.4481	25.312	0.90	5.64
3	UST-400MPa-70 °C	1:0.5	0.0076	1.00	0.02	0.7256	0.0233	0.99	0.07	0.0656	0.0823	1.00	0.01	0.0076	0.0228	1.00	0.02
		1:1	0.0296	0.99	0.14	0.6139	0.160	1.00	0.02	0.237	0.1566	0.99	0.05	0.0296	0.1926	0.99	0.14
		1:3	0.6971	0.90	8.95	0.1869	39.2013	1.00	0.02	5.684	0.4822	0.96	0.40	0.6971	40.835	0.90	8.95
4	HPP-400MPa-25 °C	1:0.5	0.0058	1.00	0.02	0.7554	0.0167	0.99	0.09	0.0568	0.0724	1.00	0.02	0.0058	0.0185	1.00	0.02
		1:1	0.006	1.00	0.01	0.9087	0.0082	1.00	0.07	0.0105	0.0769	1.00	0.01	0.006	0.0031	1.00	0.01
		1:3	0.0689	1.00	0.08	1.2362	0.0243	0.99	0.15	−0.1232	0.2754	1.00	0.07	0.0689	−0.0636	1.00	0.08
5	Thermal treatment-72 °C-15s	1:0.5	0.0025	1.00	0.001	0.8811	0.0039	0.99	0.12	0.0133	0.0483	1.00	0.01	0.0025	0.0017	1.00	0.001
		1:1	0.0034	1.00	0.005	0.8835	0.0055	1.00	0.06	0.0169	0.057	1.00	0.01	0.0034	0.0035	1.00	0.005
		1:3	0.0454	1.00	0.09	1.1600	0.0175	0.99	0.17	−0.1122	0.2195	0.99	0.08	0.0454	−0.0814	1.00	0.09
6	Homogenization-2000psi-500psi-70 °C	1:0.5	0.0031	1.00	0.003	0.8259	0.0059	1.00	0.06	0.0186	0.0543	1.00	0.00	0.0031	0.0031	1.00	0.003
		1:1	0.0045	1.00	0.003	0.7488	0.0097	0.97	0.18	0.0233	0.0644	1.00	0.01	0.0045	0.0041	1.00	0.003
		1:3	0.1143	0.98	0.60	0.4737	1.0529	0.99	0.06	0.6913	0.2859	1.00	0.08	0.1143	1.1027	0.98	0.60

shown in blue color (lesser height) in Fig. A2d. Thus, the tiny dark spots in the white region in Fig. A2a can be attributed to the milk fat–protein content. Further comparison of particle size parameters of untreated milk:pea and water:pea suspensions showed that the mean diameter and average height of milk:pea and water:pea suspensions were not significantly different (Table A1). This finding indicated that the contribution of milk fat and protein to the particle size parameters of untreated milk:pea samples were negligible.

3.7.2. Laser scanning microscope (LSM) images of treated samples

Fig. 7 shows the microstructures of milk:pea samples of 1:0.5 and 1:3 protein ratios under different treatments obtained by LSM. The untreated milk:pea 1:0.5 samples showed individual pea protein particles dispersed in the milk matrix (Fig. 7a). In untreated milk:pea 1:3 samples, the pea protein particles were linked together due to higher concentration of pea protein (Fig. 7b). There was a clear difference between the untreated milk:pea 1:0.5 samples and 70 °C UST-treated samples (Fig. 7c), demonstrating the role of UST in reducing the particle size of samples in order to make the blend stable. The 70 °C UST treatment of milk:pea 1:3 samples produced complex protein–protein or protein–fat interactions, resulting in a homogenous product (Fig. 7d). As stated earlier, the network might be a result of aggregation of small particles created by shear treatment. Formation of small particles through disruption and subsequent fragmentation increases the surface area of the particles and changes the properties of the particles and serum, to promote complex interactions (Augusto, Ibarz, & Cristianini, 2012).

Homogenization appeared to reduce the particle size in milk:pea 1:0.5 samples (Fig. 7e), but the reduction was apparently lesser than that produced by UST treatment. In milk:pea 1:3 samples, homogenization blended milk and pea protein components, though the association was different from that created by UST. The observations in microstructure (Fig. 7) corroborated the particle size measurements discussed in section 3.2.

3.7.3. Scanning electron microscope (SEM)

The microstructures of milk:pea samples of 1:3 protein ratio under different treatments obtained by SEM are given in Fig. 8. In the untreated samples, the individual pea protein particles were larger and are apparently clustered together (Fig. 8a). After 40 and 70 °C UST treatment, the pea protein particles were size reduced, dispersed, and appeared enwrapped in the milk matrix (Fig. 8b and c). There was a clear difference between the UST-treated and untreated samples, which might be attributed to the UST-induced particle disruption and subsequent fragmentation. The exposed cell constituents enable interactions between particles such as proteins and change the properties of particles and serum (Augusto et al., 2012; Kubo et al., 2013).

The HPP-treated samples showed individual pea protein particles dispersed in milk indicating that HPP did not affect the size of pea protein particles (Fig. 8d). Homogenization caused disruption of pea protein particles; however, the gel structure is different than the UST treatments (Fig. 8e). After thermal treatment, there was no change in the pea protein particle size even though the particles seemed to be aggregated together (Fig. 8f). The observations corroborated the particle measurements, sedimentation index, and rheological changes discussed in earlier sections.

3.8. Proposed pathways for blending of milk and pea protein by UST

Based on the observations in the study, the mechanism behind blending of milk and pea proteins by UST treatment can be explained by the following possibilities.

i) The high pressure, shear and temperature in UST led to size reduction of the pea protein and subsequent entrapment of the particles in the milk casein micelles. In low protein milk:pea (1:0.5 and 1:1) samples, the blended particles are disperse and

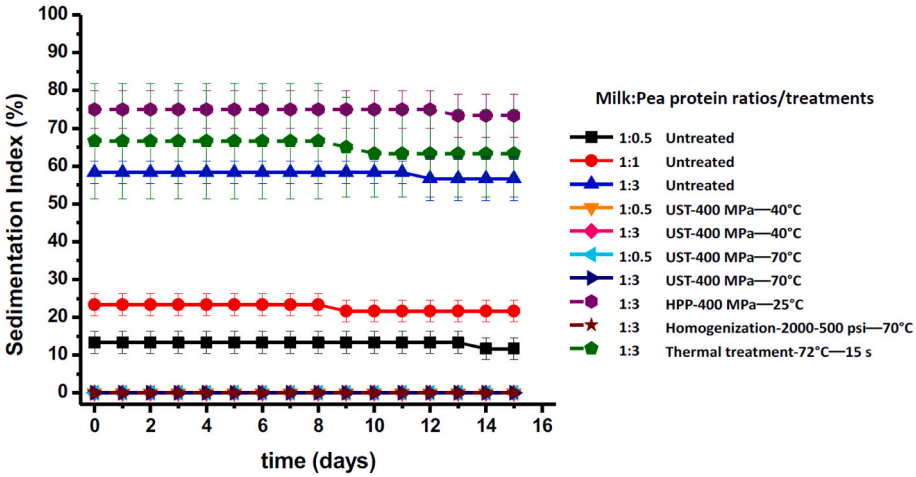

Fig. 5. Sedimentation index of test samples.

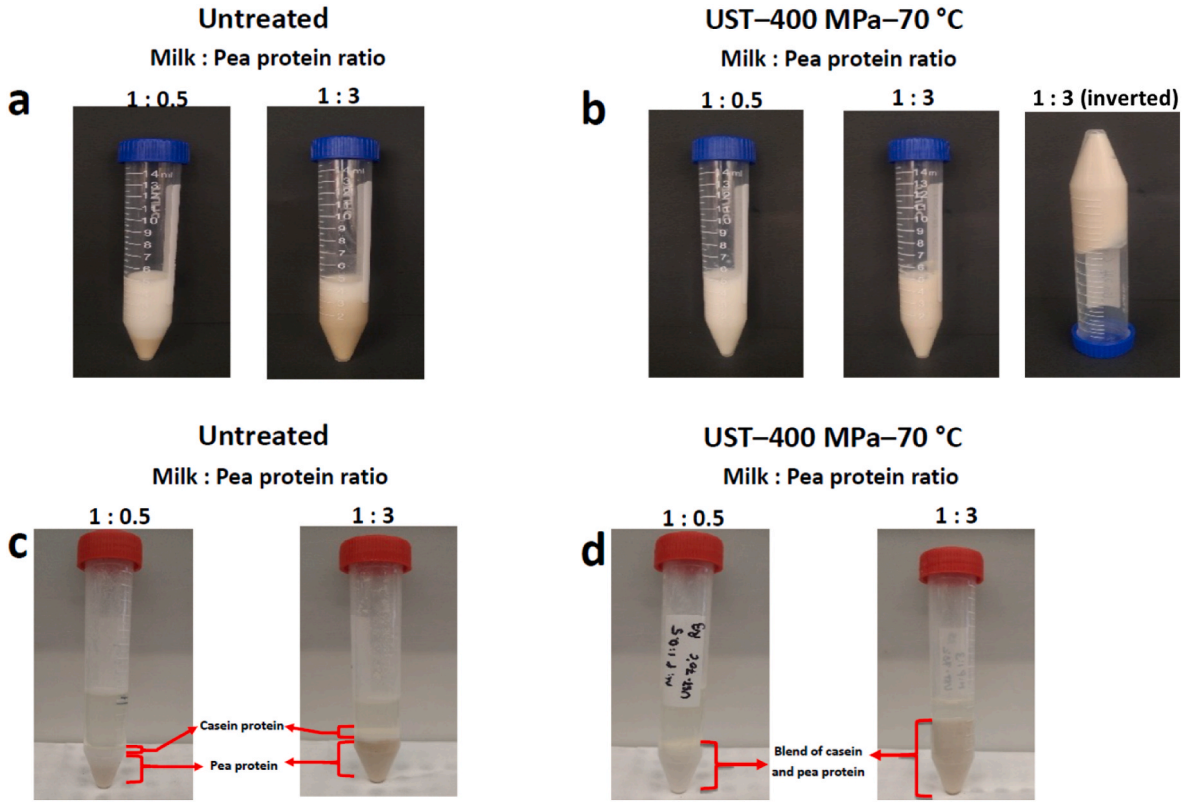

Fig. 6. Images of (a) untreated milk:pea protein 1:0.5 and 1:3 samples (b) 70 °C UST treated milk:pea protein 1:0.5 and 1:3 samples [The inverted tube with milk:pea 1:3 samples indicate the gel structure of the samples] (c) untreated milk:pea protein 1:0.5 and 1:3 samples with pH adjustment up to 4.6 (d) 70 °C UST treated milk: pea protein 1:0.5 and 1:3 samples with pH adjustment up to 4.6.

result in dilute solution. In high protein milk:pea 1:3 samples, the particles are closely packed thereby causing linkages between adjacent particles. The linkages are also promoted by fat globules. Further, the pressure associated shear and temperature during UST treatment causes partial unfolding of whey proteins in milk. The unfolded proteins aggregates to form clusters, resulting in increased viscosity and elasticity (Fig. 9a).

ii) The UST treatment disintegrated the casein micelles in milk into smaller submicelles or casein proteins. The pea protein is bonded to individual casein proteins due to pressure associated shear action resulting in stable blends (Fig. 9b).

iii) The casein submicelles or proteins initially disintegrated by UST bind the pea protein and eventually associate back to form milk-pea protein blends (Fig. 9c).

The binding of pea protein with casein protein by UST is clearly illustrated by Fig. 6d. The blending might be contributed by any one or all the possible mechanisms listed above. Future studies should focus on studying the interactions between milk and pea proteins at molecular scale to gain further insights.

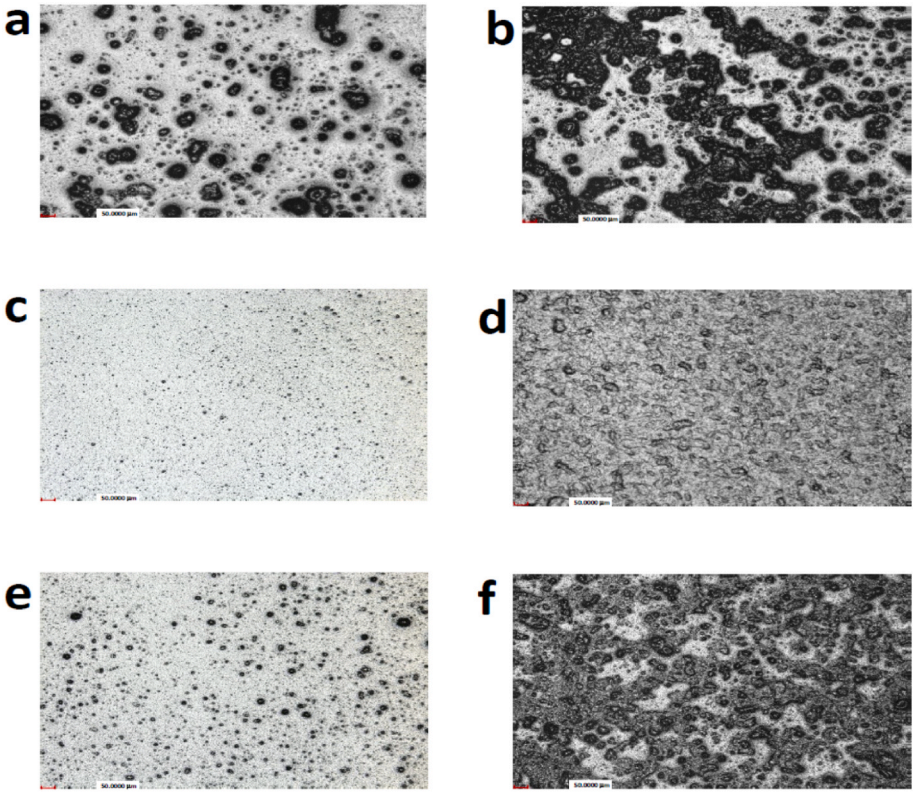

Fig. 7. Laser scanning microscopy (LSM) images at 10 × magnification (scale bar is 50 μm) showing the microstructure of (a) untreated milk:pea protein 1:0.5 samples (b) untreated milk:pea protein 1:3 samples (c) milk:pea protein 1:0.5 samples treated by UST-400 MPa-70 °C (d) milk:pea protein 1:3 samples treated by UST-400 MPa-70 °C (e) milk:pea protein 1:0.5 samples treated by Homogenization-2000psi-500psi-70 °C (f) milk:pea protein 1:3 samples treated by Homogenization-2000psi-500psi-70 °C.

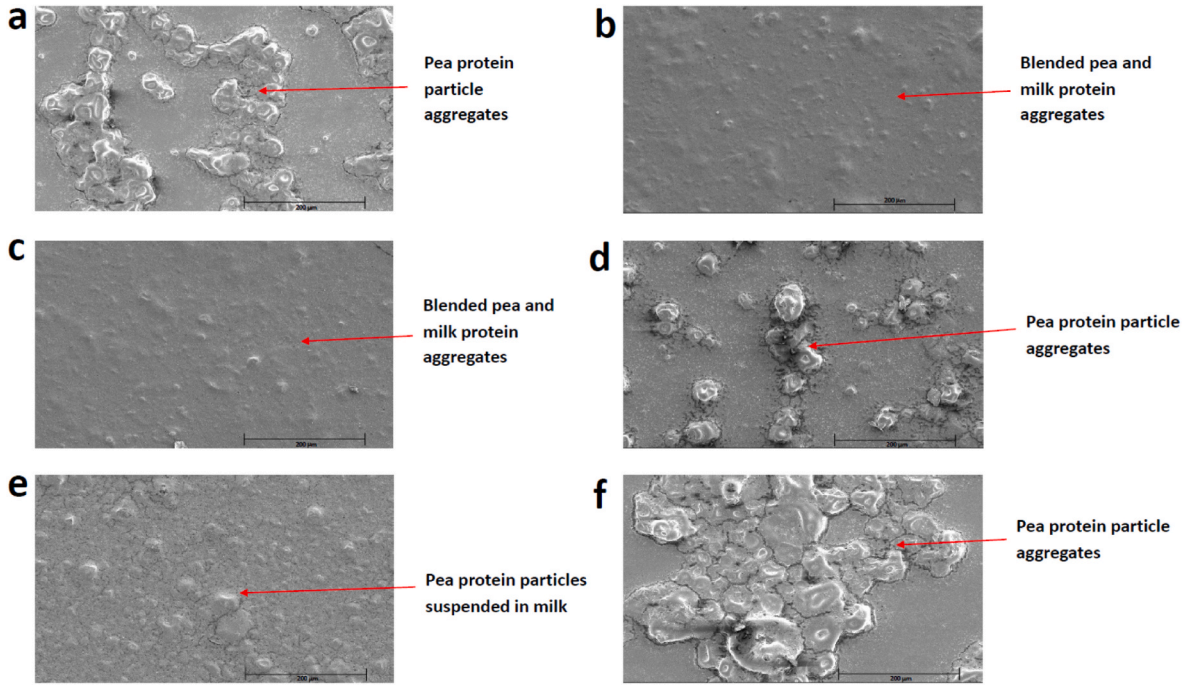

Fig. 8. Scanning electron microscope (SEM) images at 350 × magnification (scale bar is 200 μm) showing the microstructure of milk:pea protein 1:3 samples (a) untreated (b) treated by UST-400 MPa-40 °C (c) treated by UST-400 MPa-70 °C (d) treated by HPP-400 MPa-25 °C-0 min (e) treated by Homogenization-2000psi-500psi-70 °C (f) treated by Thermal treatment-72 °C-15s.

4. Conclusion

Pressure-only and thermal-only treatments did not alter the particle size and caused sedimentation of pea protein particles in the milk matrix, resulting in an unstable mixture. Interaction of pressure, shear, and temperature during UST treatment of milk–pea suspensions altered the particle size and created particle–particle interactions thus creating products of varied consistencies, depending on protein concentration, with potentially different applications. At lower milk:pea protein ratios of 1:0.5 and 1:1, the UST-treated products were stable and represent

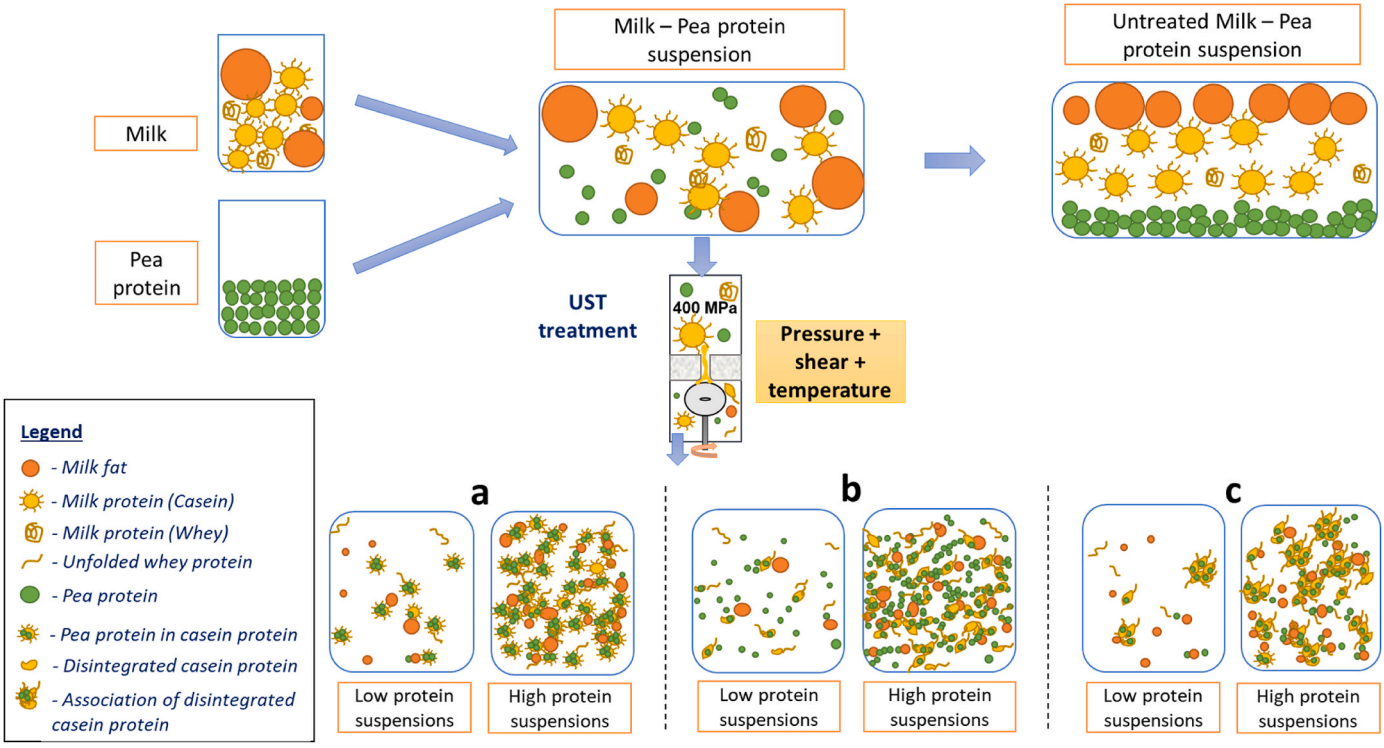

Fig. 9. Schematic representation of the proposed pathways for creation of stable milk-pea blends by UST treatment.

pea–dairy-based milk, creams, sauce-type products, and beverages. At high milk:pea protein ratio of 1:3, the UST treatment produced stable products of higher viscosity, representing pea–dairy gel-type products. Due to technical challenges, the equipment used in the study had different pressure-come up time for HPP and UST experiments. Such differences may have additional impact on the characteristics of milk-pea suspensions. Future studies from our laboratory will also consider how pressure, heat and shear influence different type of pea and milk proteins (including legumin, viclin, β-lactoglobulin, α-lactalbumin) with varied conformations.

The ability of UST to create stable products and gel networks between particles based on the initial protein concentration is identified. The protein structural changes at molecular level, the interaction between protein and non-proteinaceous components like carbohydrate, during UST treatment merits additional investigation. The UST enables clean label products due to no addition of synthetic binding agents to prevent separation of pea and milk protein components. This information would be valuable for development of milk–plant protein-based products for varied end uses. Further, the study of the rheological characteristics of the milk–pea suspensions under pressure, shear, temperature, and their interactions showed that products with unique rheological characteristics can be created by UST. This information will be useful to UST equipment engineers to design equipment components such as shear valve, hold tube, and pumps to handle the product.

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CRediT authorship contribution statement

Jerish Joyner Janahar: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft. **V.M. Balasubramaniam:** Conceptualization, Resources, Methodology, Formal analysis, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Rafael Jimenez-Flores:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition. **Osvaldo H. Campanella:** Writing – review & editing, Supervision, Methodology, Formal analysis. **Israel García-Cano:** Methodology, Formal analysis, Data curation, Writing – review & editing. **Da Chen:** Methodology, Formal analysis, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2022.107811.

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