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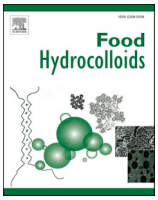

Engineering plant-based Pickering emulsions as highly stable dairy cream alternatives using a binary mixture of particle stabilisers

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ABSTRACT

Pickering emulsions (PEs) stabilised by colloid solid particles are a promising sustainable alternative to surfactant-based ones. Binary mixtures of particles with different functional characteristics can be used to design novel PEs with enhanced properties and stability. In particular, binary mixtures of differently charged particles are a powerful way to control the droplet size, coverage, and particle loading in the emulsions. In this study, we report the processing of highly stable plant-based Pickering emulsions of sunflower oil with thickened cream morphology using optimally modified oat flour and faba bean protein isolate (2,3, and 4% w/w) as a binary mixture. The colloid particles were prepared with a slight pH treatment and analysed for protein solubility, surface charge and emulsifying properties. Meanwhile, the plant-based Pickering cream (PPCs) were prepared with single particles (as control) and binary mixtures and characterised for particle size, microstructure, flow behaviour and storage stability. The PPCs prepared with a binary system exhibited exceptional attributes, including uniform and small droplet sizes ranging from 14.00 to 20.57 μm, thick consistency and smooth shear thinning behaviour. The synergistic influence of faba bean with oat flour on emulsion formation and stabilisation was found through microstructural analysis. These findings provide strong evidence for engineering highly stable plant-based PEs without the use of supplementary emulsifiers for designing sustainable and clean-label non-dairy emulsions.

1. Introduction

Pickering emulsions (PEs) stabilised by colloidal solid particles have gained substantial research attention as they offer exceptional capabilities in designing emulsions with new raw materials as ingredients. PEs have also become the pillars of sustainable and clean-label alternatives and are being widely utilised in various food systems to enhance their functional properties and shelf-life. According to the Scopus database (as accessed in October 2023), the research on PEs development for food industry applications is ever-increasing. In particular, the last decade (2013–2022) has witnessed exponential growth resulting in 890 documents, the highest being 269 in 2022 alone (Scopus, 2023) which indicates the relevancy and timeliness of addressing the demand for sustainable and custom-made food products. A wide range of solid particles have been used in previous studies to stabilise Pickering emulsions. They can vary in material composition [organic, inorganic], origin [plant, animal], morphology [spherical, rods, fibrous-like], size [nano-sized, micro-sized] and surface properties [hydrophilic, hydrophobic] (Björkegren, Nordstierna, Törncrona, & Palmqvist, 2017; Capron & Cathala, 2013; Ortiz, Pochat-Bohatier, Cambedouzou, Bechelany, & Miele, 2020; Sliwinski, Roubos, Zoet, van Boekel, & Wouters, 2003; Zhu, Zhang, Lin, & Tang, 2017). PEs offer the ability to design new versatile products with different functionalities such as emulsions in the form of low-fat products, yoghurt, creams, and gels and also allow nanoencapsulation of functional ingredients (Matos, Timgren, Sjöö, Dejmek, & Rayner, 2013; Xie et al., 2021). Further aligning with the rising demands of sustainability, and a significant dietary shift pattern towards vegan diets, plant-based ingredients are being used as Pickering stabilisers to develop novel and functional plant-based Pickering emulsions (PPEs). Plant-based solid particles offer several advantages, including versatile functionality, biodegradability, and simple surface modifications (Wang et al., 2022).

Although PPEs offer greater stability than surfactant-stabilised emulsions against coalescence and Ostwald ripening, they remain sensitive to their environmental conditions. One of the key challenges that remains for PPEs is maintaining their stability, and texture

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characteristics in a multi-component system. To resolve this issue, the addition of polymers and surfactants, such as xanthan gum and sodium dodecyl sulfate has been studied (Ji & Wang, 2023; Sharma, Kumar, Chon, & Sangwai, 2015). Nonetheless, these approaches have certain shortcomings as they are likely to cause environmental and health problems and also degenerate sensitive chemical compounds (Delorme et al., 2011; Zafeiri & Wolf, 2019). Moreover, to utilise the Pickering stabilisers, it is often necessary to modify the structural characteristics of surfactants, such as surface modification, particle wettability or chemical modifications using harsh chemicals or vigorous processing techniques (Xia, Xue, & Wei, 2021). In this scenario, PPEs stabilised by a binary mixture of particles have played a key role in posing a possible solution, that is, two or more types of particles being used as stabilisers to develop emulsions with greater functionality (Liu & Ngai, 2022). Such emulsions are becoming of great interest as a range of rheological properties, varied functionalities, and sensorial attributes can be exerted in one emulsion system without the need for additional external additives. Moreover, these systems can provide an opportunity to use binary mixtures with particles of different natures, such as different physicochemical properties, to enhance their functionality. According to Liu and Ngai (2022), the interactions between two types of particles in a binary mixture such as electrostatic and hydrophobic interactions, can reinforce the interface between different phases (droplet and media) in the emulsion, thus, resulting in emulsions with superior stability. In past research, unmodified pea protein-starch mixtures were used directly as Pickering stabilisers to investigate the heat-induced gelation of the emulsions (Sridharan, Meinders, Sagis, Bitter, & Nikiforidis, 2022). It was concluded that these mixtures could provide additional benefits with their binary role of using starch as a gelling agent and protein particles as emulsifiers. Despite its potential significance, this is still a very niche research area and is yet to experience extensive investigation, particularly on utilising unmodified and minimally processed plant-based ingredients for stabilising PPEs.

The current study emphasises utilising plant-based solid particles as stabilisers, specifically, oat flour and faba bean protein. We investigated a binary system of oat flour with a protein-rich source, i.e., faba bean protein to prepare a sustainable, and clean-label food emulsion, *plant-based Pickering cream* (PPC). As described by McClements (2020), plant-based cream is an oil-in-water emulsion comprising a high-fat content (30–40%) dispersed in a continuous water phase. The multi-component system of oat flour with a protein concentrate in the form of cream morphology could offer additional advantages due to its binary role (Sridharan et al., 2022).

Our effort in addressing these challenges involves modifying the plant-based ingredients at minimal energy and chemical consumption without compromising on any significant health and nutritious compliance. This has been reflected in our previous work where oat flour (OF) was utilised as PE stabiliser after minimal processing (pH-shift) (Rawal, Annamalai, Bhandari, & Prakash, 2023). The pH-shift treatment enhanced the emulsification and functional properties of wholesome oat flour for PE stabilisation, thus utilising both the soluble and insoluble particles to stabilise the emulsion. Building upon preliminary findings, the current study has aimed to modify and further enhance the emulsion properties, along with other plant proteins as a multi-component system. It is hypothesised that enhanced interactions between oat starch with other plant proteins will enhance our understanding of starch-protein-oil interactions and engineer the microstructure for improving the functionalities of PPEs. This approach offers an additional advantage of the binary role of proteins acting as emulsifying agents, forming a barrier around the oil droplets to lower the interfacial tension, and starch being dispersed in the continuous phase, acting as viscosity enhancers and bulky barriers between the oil droplets (Aluko, Mofolasayo, & Watts, 2009). Thus, this research takes one step forward toward enriching food products with added plant proteins, enhancing the sustainability aspect as well as nutritional value.

Faba bean is a protein-rich legume and a sustainable crop as it reduces the need for inputs through nitrogen fixation (Lizarazo et al., 2014). It contains bioactive constituents with health-promoting benefits (Martineau-Côté, Achouri, Karboune, & L'Hocine, 2022). These particulars indicate that it can be well-utilised as an emulsifier to develop novel and nutrient-rich plant-based food systems. However, its unfavourable emulsifying properties at an unmodified stage and low water solubility make it an underutilised ingredient in food systems. Several modification techniques have been carried out, such as mechanical, enzymatic, and chemical, to improve the functional properties of faba bean protein (FB) (Liu, Pei, & Heinonen, 2022). Aligning with the goals of our study of minimal processing and less use of harsh chemicals, a simple technique studied previously that resulted in enhanced functional properties of FB was the mild chemical treatment of changing its pH to acidic conditions (Felix, Cermeño, & FitzGerald, 2019). FB showed 19% more protein solubility, smaller droplet sizes and better rheological properties at pH 3.0 than at pH 5.0. Moreover, faster protein adsorption was noted at acidic pH (pH 3.0) when the interfacial viscoelastic properties of the FB layer in O/W emulsions were studied (Felix, Cermeño, & FitzGerald, 2019).

A few studies have used a binary mixture of particles to improve their functional characteristics through various methods such as complexation (Sun, Zhao, Liu, Li, & Li, 2019), altering pH (Li et al., 2019) and mixing solutions of oppositely charged biopolymers (Zhang et al., 2019). Altering the pH of individual particles enhances the electrostatic interactions and hydrogen bonds, which further increases the hydrophobicity and stability of the resulting system. Aligning with the objectives of our research of minimal processing, and designing clean-label PEs, the concept of altering the pH to enhance the functional properties and stability of the system was incorporated in this study. As concluded in our previous research, OF exhibited remarkable functional characteristics at basic pH (Rawal et al., 2023). With FB exhibiting good functional characteristics at pH 3.0, the idea of having a mixture of binary colloidal particles with enhanced functional characteristics (altered pH) was undertaken in this study. It was hypothesised that both faba protein and oat flour having better emulsifying and protein solubility at this definite pH would exhibit higher stability during storage and distribution.

To our knowledge, the use of binary components of oat flour and faba bean protein to prepare PPC without the use of any external surfactants has not been studied previously. Therefore, the main objective of this study is to utilise a binary mixture of oat flour and faba bean protein as Pickering stabilisers to prepare plant-based creams with varying pH and protein concentration conditions. The Pickering stabilisers, OF and FB were analysed based on surface charge, protein solubility and emulsifying properties. To study the functional properties of the PPCs, they were characterised based on particle size, microstructure, storage stability and flow behaviour.

2. Materials and methods

2.1. Materials

Oat flour (OF) was obtained in powder form as a commercial product purchased from McKenzie's Foods, Australia. The product was prepared from 100% oat kernels and consists of carbohydrate (63.9%), protein (10.9%), dietary fibre (6.6%), total fat (8.2%), and calories (13615 kJ) as provided by the manufacturer. Faba bean protein isolate (FB, Australian Plant Proteins Pty Ltd., Victoria, Australia) contained 88% protein. Sunflower oil, used as the oil phase, was purchased from MOI International Pty Ltd. (Queensland, Australia). All the chemicals used (HCl 1N and NaOH 1N) were of analytical grade obtained from Sigma-Aldrich, New South Wales, Australia.

2.2. Preparation of dispersions

Prior to dispersion preparation, the pH dependence of OF and FB solubility was investigated by dispersing separately at different pH and

measuring zeta potential. At pH 10 and pH 3, the OF and FB were found to disperse very well, respectively. Based on this observation, four sets of samples were prepared:

I. To prepare the mixture of 'OF' and FB **at pH 7.0**, 3 sets of OF (10% w/w) dispersions were prepared in deionised water with an overhead stirrer for 3 h at 500 rpm at room temperature. After optimal mixing of oat flour was ensured, FB was added to it in varying concentrations to make up the total protein content of the samples as *2, 3 and 4% w/w*. These samples were stirred for another hour under the same environmental conditions. These dispersions will be further denoted as ***OF* + *FB2, OF* + *FB3 and OF* + *FB4***, respectively.

II. To prepare the mixture of OF and FB at **varying pH,** a similar methodology as 2.2. I. was followed except that in the initial step, during the preparation of OF dispersion, the pH of the OF dispersion was adjusted to pH 10.0 with 1N NaOH and on the other hand, the pH of the FB dispersions (prepared in deionised water) was adjusted to pH 3.0 with 1N HCl. These mixtures were then mixed with an overhead stirrer for 1 h at 500 rpm at room temperature with a final pH of 5.30. These dispersions will be further denoted as ***OF'*+ *FB'2, OF'*+*FB'3 and OF'*+*FB'4*.**

III. Oat flour control samples (Control 1): Oat flour (10% w/w) dispersion was prepared in deionised water at pH 7.0 following the same protocol as mentioned in 2.2.I without FB, by mixing well with an overhead stirrer for 3 h at 500 rpm at room temperature, denoted as ***OF*.** Then another OF dispersion was prepared at *pH 10*, by adding 1N NaOH, as mentioned in 2.2.II denoted as ***OF'*.**

IV. Faba bean control samples (Control 2): For controls of faba bean, varying concentrations of FB powder (2,3 and 4% w/w) were mixed in deionised water at pH 7.0 following the same protocol as mentioned in 2.2. I without OF, by mixing well with an overhead stirrer for 3 h at 500 rpm at room temperature. The second set of control dispersion was prepared by mixing in deionised water and then decreasing pH using 1N HCl (to *pH 3.0*). All the dispersions were prepared by further mixing well with an overhead stirrer for 3 h at 500 rpm at room temperature and the samples denoted as ***FB'2, FB'3, and FB'4*.**

2.3. Preparation of plant-based pickering creams (PPCs)

Oil-in-water Pickering emulsions containing 40% sunflower oil were prepared (Keeping the oil: solid content ratio constant at 4:1) as shown in Fig. 1. The emulsions were pre-homogenised using Ultraturrax at 8000 rpm for 3 min at room temperature. Further, the samples were homogenised with twin panda homogeniser (GEA Twin Panda 400, GEA Process Engineering Pty. Ltd, Victoria) at 100 bars for two passes. Preliminary trials were done with different oil: oat ratios and same conditions were followed as previous work (Rawal et al., 2023). After homogenisation, the samples were stored at 4 °C for 24 h and analysed the next day.

2.4. Characterisation of dispersions

2.4.1. Zeta potential

To study the electrochemical interactions between the components, the dispersions of oat flour and faba bean protein individually were analysed for zeta potential that was measured by employing the Zetasizer (Malvern Zetasizer Nano ZS, Malvern Panalytical Ltd., UK) with dynamic light scattering techniques (DLS) by modifying the protocol by Shahbal, Jing, Bhandari, Dayananda, and Prakash (2023). Both oat flour and faba bean protein dispersions were diluted 100-fold with Milli-Q water and the pH was fixed to pH 3.0, 7.0 and 10.0 by altering with 0.1N NaOH or 0.1N HCl accordingly. Preliminary trials were done over a range of pH (2.0–12.0) and these pH conditions were reported as they were relevant to the study. The measurements were done in three replicates for each experimental sample at 25 °C using the zeta potential cell DTS1060.

2.4.2. Protein solubility

The protein solubility was measured according to the method described by Malomo, He, and Aluko (2014) with slight modifications. The prepared dispersions were centrifuged at 10000×*g* for 20 min at 25 °C. The protein solubility was expressed as the percentage ratio of the

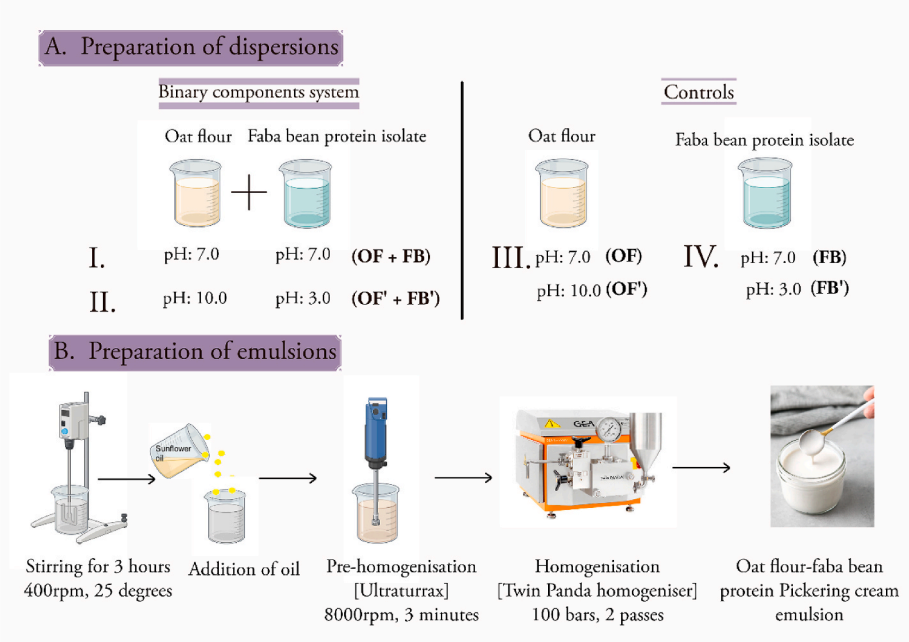

Fig. 1. Schematic diagram representing the preparation of Pickering cream emulsions using controls (oat particles and faba bean protein) and binary components as stabilisers.

supernatant protein content to the total protein content. The protein content of the dispersions was measured using the Kjeldahl analysis method (N × 6.25).

2.4.3. Emulsifying properties

The emulsifying properties can be evaluated in the form of two parameters: emulsifying activity index (EAI) and emulsion stability index (ESI) which were studied according to the method of Cruz-Solorio, Villanueva-Arce, Garín-Aguilar, Leal-Lara, and Valencia-Del Toro (2018). 0.1 g of the oat flour or faba bean protein isolate was mixed with 10 mL of MilliQ water. The dispersions were adjusted to pH 7.0, 3.0 (for faba, FB') and pH 10.0 (for oat flour, OF'). To this, 10 mL of sunflower oil was added, and the mixture was homogenised using Ultraturrax at 12,000 rpm for 1 min 50 μL of the resulting emulsion was added to 5 mL solution of sodium dodecyl sulfate (0.1% w/v). The absorbance was measured at 500 nm using the spectrophotometer. The EAI and ESI were assessed with the following equations:

$$\text{EAI}\ \left(m^2/g\right) = \frac{2 \times 2.303 \times A_0}{0.25 \times \textit{protein weight}\ (g)}$$

$$\text{ESI (minutes)} = \frac{A_{10} \times \Delta t}{\Delta A}$$

Where A_0: absorbance at 0 min after homogenisation, A_{10}: Absorbance at 10 min after homogenisation, Δt: 10 min, ΔA: A_0- A_{10}

2.4.4. Dynamic interfacial tension measurements

The dynamic interfacial tension measurements have been described in detail previously (Kontogiorgos & Prakash, 2023). The adsorption process of oat flour (OF), faba bean protein (FB), and the mixture of particles (OF + FB) was studied at the oil-water interface. The sample concentrations of 0.01 mg/mL were dispersed in 100 mM TRIS buffer and 100 mM NaCl at pH 7.2. A glass Hamilton syringe (DS500, GT) equipped with a stainless-steel needle with a 1.6 mm outer diameter (SNP 160/119) was used in the experimental setup. Dynamic interfacial tensions of the samples were monitored for 30 min using axisymmetric drop shape analysis with a pendant drop tensiometer (OCA-15EC, DataPhysics Instruments, Germany) using the Young-Laplace fitting. All measurements were performed in triplicates, and average values are reported.

2.5. Characterisation of the pickering creams (PPCs)

2.5.1. Particle size distribution

The particle size distribution of the emulsions was determined using the laser light scattering particle sizer (Mastersizer 2000; Malvern Instruments, Malvern, UK) at 2000 rpm. The emulsions were gently inverted 5 times and added dropwise to water until the laser obscuration was around 12%. The refractive indices of water, oat flour and oil were 1.33, 1.335 and 1.47, respectively. The parameters of the volume-weighted mean ($D_{4,3}$), surface-weighted mean ($D_{3,2}$), and span [(d (0.9)-d (0.1))/d (0.5)] were obtained from the instrument software and reported as the mean of three measurements of each batch of emulsion.

2.5.2. Flow behaviour

The flow behaviour properties of the emulsions were characterised using Rheometer (ARG1500, TA Instruments, New South Wales) using steady-state measurement with a 60 mm plate cone plate geometry (cone angle: 2°). The measurements were done at 25 °C, and the shear rates ranged from 0.01 to 100 s^{-1}.

2.5.3. Microstructure

To gain knowledge about the physical arrangements of particles, the microstructure of the PPCs was observed under the Confocal Laser Scanning Microscope (Zeiss LSM 700). The emulsions prepared with OF and a mixture of OF and FB were stained with 10 μL of Rhodamine B (0.005% w/w in distilled water, for protein) and 20 μL of fluorescein 5-isothiocyanate (FITC, 0.2% w/w in distilled water, for starch). The FB-based emulsions were stained with approximately 10 μL of Nile Red (0.02% w/w in PEG200, for fat) and 10 μL of Rhodamine B (0.005% w/w in distilled water, for protein). After staining with the dyes, the samples were left standing for 2 min to let the dyes penetrate the samples. Following that, 0.4 μL of the sample was pipetted onto the slide and a coverslip was placed. The excitation for protein, starch and fat phase was set at 555 nm, 488 nm, and 488 nm, respectively (Auty, Twomey, Guinee, & Mulvihill, 2001; Velde, Riel, & Tromp, 2002).

2.5.4. Storage stability

The storage stability of the OPEs was measured against creaming in accordance with the Keowmaneechai & McClements, 2002 method. 20 mL of the samples were enclosed in 30 mL containers, stored at 4 °C, and monitored for 21 days. Gradually, the separation occurred, forming an upper cream layer and a lower serum layer. The creaming index (%) was calculated for the 21st day using Equation (1):

$$CI\ (\%) = \left(\frac{H_S}{H_T}\right) X\ 100 \quad (1)$$

CI: Creaming index; H_S: Height of the serum (cm, Height of transparent/turbid layer of the vessel); H_T: Total height of the emulsion (cm)

2.6. Statistical analysis

Three replications were performed, and the data were reported as mean values of the triplicates with the standard deviations. The data were analysed by analysis of variance (ANOVA) with a confidence level of 95% using the software SPSS (Version 29.0) and GraphPad Prism (Version 9.5.1). In addition, Tukey's test was performed for multiple comparisons of the mean values with a 5% level of significance (P-value$<$0.05).

3. Results and discussion

3.1. Characterisation of dispersions

Protein solubility, surface charges and emulsifying properties are important factors in studying emulsion stability as these systems are stabilised by a combination of steric and electrostatic repulsions (Felix, Lopez-Osorio, Romero, & Guerrero, 2018). The results obtained from analysing the dispersions are presented below.

3.1.1. Zeta potential

Zeta potential (ZP) gives a measure of the net charge on the surface of the particle and of the distribution of the electric potential at the interface (Parupudi, Mulagapati, & Subramony, 2022). It is the best indicator for the stability of the dispersions as it is important to understand the electrochemical interactions between the food elements since they affect the texture, flavour, structure, and other essential characteristics of the final food system (Cano-Sarmiento et al., 2018; Solanki et al., 2024). The amphiphilic nature of proteins allows them to be present in the aqueous phase and adsorb at the oil droplets, simultaneously, thereby generating stabilising forces and steric interactions. The net charge on the sample should be in balance, i.e. large enough to overcome the several attractive forces (such as van der Waals and hydrophobic) and also result in significant repulsive forces between oil droplets (McClements, 2004). Food components, specifically, proteins exhibit a negative net charge when the pH is lower than their isoelectric point (pI) and a positive net charge when the pH is above the pI. As a result, pH is one of the major factors that influence the surface charge of materials, which subsequently impacts the equilibrium between the protein-solvent (hydrophilic) and protein-protein (hydrophobic) interactions (Guo, Tian, & Wu, 2019). In this study, acidic (3.0), neutral

(7.0), and basic (10.0) pH conditions were studied that would help us understand, (i) the behaviour of OF and FB with changing pH, and (ii) the effect of their surface charge on the emulsifying properties to prepare PPCs.

The ZP profile of both samples is presented in Fig. 2. For OF, the ZP varied from 2.35 mV (pH 3.0) to −33.28 mV (pH 10.0). At pH 10.0, OF showed a ZP value of −33.28 mV, which indicated good physical colloidal stability due to sufficient repulsive forces (Beliciu & Moraru, 2011). This can be explained by an increase in the net charge of proteins on moving away from the isoelectric point (pI) of oat proteins (pH 5.0), towards an alkaline environment (Li & Xiong, 2021). Additional information on the colloidal dispersions' stability for OF and OF' has been explained in our previous study with the particle size, zeta potential, scanning electron microscopy and protein solubility analysis. The particle size analysis exhibited that the droplet diameter of OF' (1.34 ± 0.01 μm) was significantly more than the OF (1.24 ± 0.04 μm), which could be due to the swelling of starch particles in the alkaline environment (Rawal et al., 2023).

Meanwhile, FB showed a similar curve as OF, wherein the ZP values ranged from 28.14 mV (pH 3.0) to −40.12 mV (pH 10.0) (Fig. 2). A crossover at the isoelectric point (for FB: 5.0–5.5) occurred wherein the ZP was 0 mV (Results not shown). A high value of zeta potential, such as at pH 3.0 (28.14 mV) promotes the hydration of proteins and induces high repulsive interfacial charges (Schwenke, 2001). In a previous study, FB exhibited smaller droplet sizes, and better rheological characteristics at pH 3.0 than at basic conditions that were related to the protein unfolding (Felix, Cermeño, & FitzGerald, 2019). The net negative charge for FB at neutral and alkaline conditions was due to the exposure of hydrophobic parts of the protein side chains of aspartic acid and glutamic acid residues spatially located on the surface of the protein (Liu et al., 2022).

As explained in the introduction (Section 1), after gathering an understanding of the behaviour of surface charge over a range of pH for both the individual components, OF and FB, it was decided to mix them at different pH to form a binary system with improved functional characteristics. In the case of OF, at pH 10.0, oat proteins exhibited improved functional characteristics, similar to FB which showed improved solubility and emulsifying properties at pH 3.0 (Fig. 3). This information will help gain a better understanding of how oat flour would behave with another protein source and prepare a PE stabilised with binary solid particles with improved functional properties.

3.1.2. Protein solubility and emulsifying properties

The emulsifying ability of the ingredients is one of the crucial functional characteristics of food ingredients, as emulsions are quite common systems to exist in several aqueous foods (McClements, 2016). In the emulsions, protein solubility is an important physicochemical property as it affects other functional properties in the food system, such as the texture, emulsifying, foaming, and gelation properties (Rawal, Annamalai, Ningtyas, & Prakash, 2024). Also, the solubility of the ingredients affects the emulsification by facilitating the migration of solid particles to spread at the oil-water interface. The ability of proteins to solubilise depends on the balance between the protein-protein (hydrophobic) and protein-water (hydrophilic) interactions (Wu, Hettiarachchy, & Qi, 1998). Since good solubility of proteins is a prerequisite for enabling access to functionality and emulsifying properties, we investigated the interrelationship between the solubility, and emulsifying properties of the OF, FB and binary colloidal particles. Fig. 3a shows the protein solubility of the dispersions.

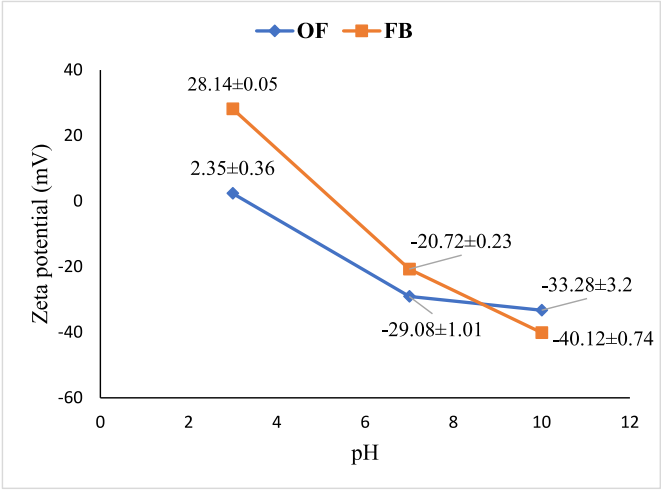

Fig. 2. Zeta potential of oat flour and faba bean protein at pH 3.0, 7.0 and 10.0.

Emulsifying properties can be expressed in terms of (i) Emulsifying activity index (EAI) which determines the amount of oil that can be emulsified per unit protein; and (ii) emulsion stability index (ESI) which measures the resistance of the emulsion to undergo any changes (flocculation, coalescence) over a specific time (Aryee, Agyei, & Udenigwe, 2018). The emulsifying properties of FB and OF are presented in Fig. 3b. OF showed solubility of 17.92% which significantly improved at pH 10 for OF' (30.42%) (Fig. 3a. I). This was attributed to the partial unfolding of the proteins due to intermolecular side-chain charge repulsions. This effect results in a reduction of hydrogen bonds, disrupting the hydrophobic interactions and improving the solubility of oat proteins at alkaline pH (Kumar, Sehrawat, & Kong, 2021). Previous research studies reported that at alkaline pH, the solubility and emulsifying properties of oat proteins improved exceptionally (Ma & Harwalkar, 1984). The results were in agreement with the EAI and ESI values that significantly improved for OF' (183.10 m^2/g, 112.20 min) than OF (49.01 m^2/g, 22.60 min), respectively; exhibiting a direct relation between the solubility and emulsifying characteristics of oat flour dispersion. Additionally, the droplet size for OF' dispersion was marginally more.

In the case of faba bean protein, FB2 exhibited protein solubility of 36.53%, which decreased with an increase in protein concentration to 19.21% (FB3) and 14.50% (FB4) (Fig. 3a. II). However, at pH 3.0, the samples FB'2 (41.86%), FB'3 (51.35%), and FB'4 (49.78%) showed a significant increase in solubility than their counterparts. FB contains about 80% globulin, which in turn constitutes of majorly legumin and vicilin. Legumin, at acidic pH, exhibits enhanced water solubility (Warsame, Michael, O'Sullivan, & Tosi, 2020). This may be related to its dissociation into lower molecular weight subunits and low ionic strength at acidic pH (Koyoro & Powers, 1987). Similar results were supported with the emulsifying properties' data for FB at acidic pH (FB') exhibiting significantly more EAI (162.00 m^2/g) and ESI (55.80min) than at neutral pH (FB) with 137.80 m^2/g (EAI) and 16.58 min (ESI). Moreover, Alavi, Chen, Wang, and Emam-Djomeh (2021) concluded in their research that at pH 3.0, the faba bean protein, with better emulsifying properties (than at pH 6.0), the emulsions exhibited excellent stability against flocculation. Thus, it can be inferred that oat flour at pH 10.0 and faba bean protein at pH 3.0 exhibited improved EAI, ESI and protein solubility, thus, enhancing the functional characteristics of the resulting emulsions. When mixed together, OF-FB showed an intermediate protein solubility trend, somewhere between both the individual components (Fig. 3a.III). Similar to the previous trend, the solubility decreased with an increase in protein content with OF + FB2, OF + FB3 and OF + FB4, exhibiting solubilities of 20.5%, 15.7% and 10.39%, respectively. However, in the case of a binary system with altered pH, there was a marginal decrease in the protein solubility with an increase in protein concentration following 24.86% (OF'+FB'2), 17.25% (OF'+FB'3), and 16.63% (OF'+FB'4). Interestingly, OF'+FB'4 had significantly more protein solubility (16.63%) than its counterpart at neutral pH, which exhibited 10.39% solubility (Fig. 3a.III).

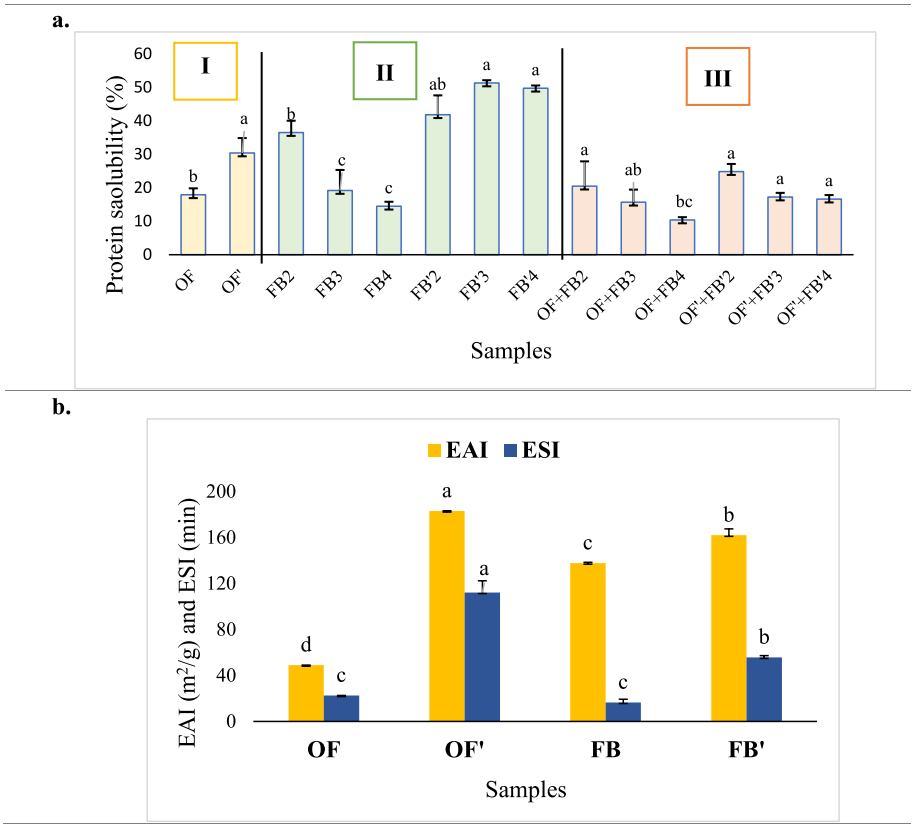

Fig. 3. **(a)** Protein solubility of the prepared dispersions (OF and FB) capturing the effect of change in protein concentration and pH. Black solid lines signify the comparison between the means of those samples using Tukey's test represented by letters a, b, and ab; **(b)** Emulsifying activity index and emulsifying stability index for oat flour (at pH 7.0, 10.0) and faba bean protein (at pH 7.0, 3.0). Tukey's test was used for the comparison of means between EAI and ESI values individually.

3.2. Characterisation of emulsions

3.2.1. Particle size distribution

To investigate the functional characteristics of the prepared PPCs, particle size distribution (PSD) was measured by the laser light particle size analyser. Emulsions' droplet size plays an important role in emulsion stability since it influences its creaming rate, coalescence, microstructure, and rheology (Santos, Trujillo-Cayado, Carrillo, López-Castejón, & Alfaro-Rodríguez, 2022). The particle size parameters, $D_{4,3}$ and $D_{3,2}$ values represent the ratio of large and small particles in the system. These parameters directly affect the physical stability of the emulsion since the large droplet sizes lead to a lower interfacial layer that consequently results in poor emulsion stability. Normally, smaller medium droplet sizes and lower span (narrow size distribution) provoke enhanced physical emulsion stability (Al-Malah, Azzam, & Omari, 2000; Camacho-Lie, Antonio-Gutiérrez, López-Díaz, López-Malo, & Ramírez-Corona, 2023).

Fig. 4 showed the variation of volume (%) with particle size of the samples by capturing the effect of change in protein concentration and change in pH of the (i) PPCs prepared with controls, OF (Fig. 4a) and FB (Fig. 4b), (ii) PPCs prepared with binary components at neutral and altered pH conditions (Fig. 4c). Table 1 shows the particle size distribution data [$D_{4,3}$, $D_{3,2}$, d (0.9) and span] obtained for all the emulsions.

Comparing OF and OF', the $D_{4,3}$ decreased significantly from 32.81 μm to 16.48 μm as per Tukey's test of comparison (Table 1). A possible explanation could be the increase in pH that improved the oat protein solubility and emulsifying properties (Mel & Malalgoda, 2021). The EAI and ESI data supported this (Fig. 3b). The improvement in emulsifying properties at alkaline pH lowered the interfacial tension, which enhancedshe emulsion stability for OF'-based emulsion. Moreover, the surface-weighted mean ($D_{3,2}$) value was significantly lower for OF' (6.59 μm) than OF (14.35 μm) and 90% of the particles were below 51.71 μm (OF) and 21.05 μm (OF') (Table 1).

Fig. 4b shows the PSD profile for FB-based emulsions as a function of protein concentration and pH. FB under neutral conditions (FB2, FB3, FB4) showed multimodal curves. The droplet size peaks ranged from 360 nm to 69.18 μm (FB2), 60 nm to 45.70 μm (FB3) and 300 nm to 831.76 μm (FB4). FB3 (9.70 μm) and FB4 (14.51 μm) showed significantly smaller droplet sizes ($D_{4,3}$) than FB2 (35.03 μm) (Table 1). However, FB4 exhibited a multimodal peak with a small tail of droplet sizes in the range of 79.43 μm–831.76 μm (Fig. 4b) which may be due to the formation of aggregates of protein and fat particles. A similar trend was observed in a study wherein the homogenisation of emulsions with higher protein concentration led to the denaturation of the proteins leading to a decrease in solubility, and protein and oil droplets aggregation (Keivaninahr, Gadkari, Benis, Tulbek, & Ghosh, 2021). This behaviour of FB4 is also supported by an increase in the value of $D_{3,2}$ and span (4.47 μm, 2.99) as compared to FB3 (2.78 μm, 2.13), respectively (Table 1). Overall, 90% of the particles were below 50 μm for all the FB-based emulsions at pH 7.0 (Table 1). At pH 3.0, the droplet size significantly decreased for all the FB-based emulsions followed by 22.58 μm (FB'2), 4.37 μm (FB'3) and 4.58 μm (FB'4) as compared to their counterparts FB2 (35.03 μm), FB3 (9.70 μm), and FB4 (14.51 μm), respectively. Previous studies have shown that at pH 3.0, smaller droplet sizes were obtained and protein adsorption around the oil droplets took place significantly faster at pH 3.0 for faba bean protein than at basic conditions due to the unfolding of the proteins at the interface (Felix et al., 2018; Felix, Romero, Carrera-Sanchez, & Guerrero, 2019). This is in agreement with EAI and ESI data (Fig. 3b).

Interestingly, the mixtures of FB and OF at neutral conditions exhibited an intermediate behaviour of droplet sizes between that of OF and FB-stabilised emulsions, individually. The $D_{4,3}$ showed a decreasing

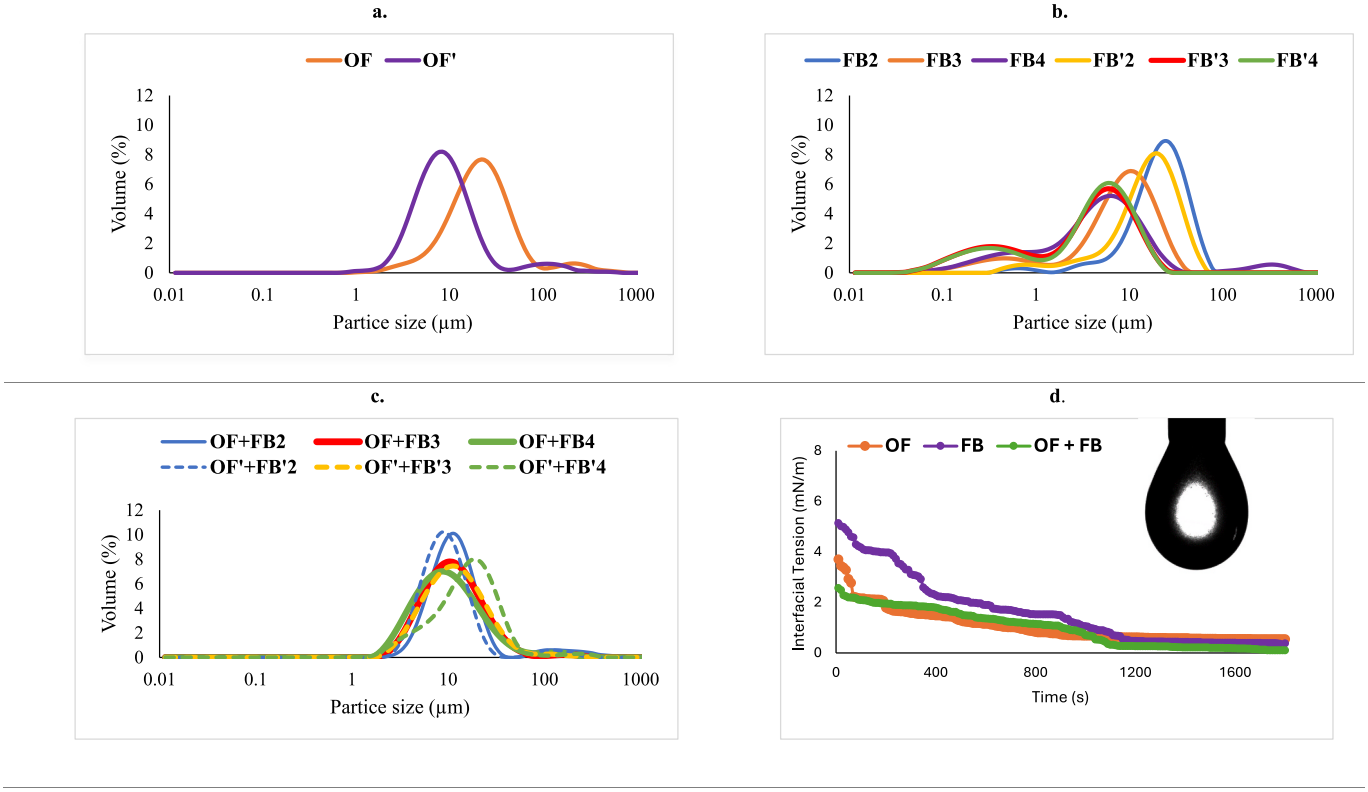

Fig. 4. Particle size distribution plots for Pickering creams prepared using, (a) oat flour (OF and OF'), (b) faba bean protein (FB and FB'), and (c) Binary system of oat flour and faba bean protein (OF + FB, OF'+FB'); (d) Dynamic interfacial tension of the oil-water interface stabilised with oat flour (OF), faba bean protein (FB) and binary system of oat flour and faba bean protein (OF + FB) with inset showing a macroscopic image of the pendant drop formed by the binary mixture of particles.

trend from 20.23 μm (OF + FB2) to 19.25 μm (OF + FB3) and 14.00 μm (OF + FB4), respectively, as the protein concentration increased. Similar results were reported by Aziz et al. (2020) wherein the effect of protein and oil volume concentrations was studied on the emulsifying properties of the acorn protein isolate. There was a significant decrease in $D_{4,3}$ and $D_{3,2}$ values as the protein concentration increased. A similar trend was observed in the case of PPCs prepared with altered pH conditions but with smaller emulsions droplets than their counterparts (Table 1). As explained by Taneja, Ye, and Singh (2015), at a given protein concentration and homogenisation conditions, the emulsifying ability of the protein is determined by its emulsions' average droplet diameter. With a better emulsifying activity and stability index of the individual components at their respective pH (Fig. 3b), the components together formed smaller droplet sized emulsions wherein the particles are stabilising the emulsion by forming a randomly packed monolayer at the interface of the two phases (Liu & Ngai, 2022). Additionally, the droplet size decreased marginally from 15.89 μm (OF'+FB'2) to 14.00 μm (OF'+FB'4) as the FB protein concentration increased. Proportionately, the amount of bigger particles decreased and the amount of small particles increased (Hauptmann et al., 2018). The smaller droplets are indicative of the superior emulsifying ability of FB.

3.2.2. *Flow behaviour*

Flow behaviour properties of emulsions serve as a useful tool as they give an insight into the interactions between the components of the emulsion system (Goodarzi & Zendehboudi, 2018). The flow behaviour properties of all the emulsions are presented in Fig. 5 by the plot of apparent viscosity (Pa.s) versus shear rate (s^{-1}). All the Pickering creams exhibited pronounced shear thinning behaviour as the viscosity decreased with an increased shear rate. According to a previous study, emulsions with high fat content, such as creams, tend to show shear thinning behaviour that is closely related to the droplet aggregation in the suspension. With an increase in shear rate, the hydrodynamic forces could become large, which leads to the deformation of the flocs that eventually disrupt the shear flow field, thus reducing the viscosity (Zhu, Ga, Liu, Zou, & McClements, 2019). To draw a further comparison, the apparent viscosity at $50s^{-1}$ (η_{50}) was compared for all the emulsions and is presented in Table 1. The apparent viscosity at this shear rate has been suggested to have a good correlation with the perceived thickness, stickiness, and sliminess for a variety of food products from Newtonian fluids to thick emulsions (Nguyen, Kravchuk, Bhandari, & Prakash, 2017; Wood, 1968).

Comparing the viscosity of the PPCs formed with oat flour, the apparent viscosity of OF' (30.67 Pa.s) is significantly higher than OF (13.63 Pa.s) at 0.01 s^{-1} shear rate (Fig. 5a). This may be due to the increased swelling of starch in oat flour at an alkaline pH that resulted in more uptake of water by the swollen starch, thus making the continuous phase thicker (Chou et al., 2020). Similar results were obtained by Niu, Pu, Li, Ma, and Hu (2017) to study the solvent retention capacities of oat flour. In the case of FB-based PPCs (Fig. 5b), a higher concentration of proteins significantly increases the apparent viscosity of the emulsions. The reason for this phenomenon could be that a higher concentration of protein increases the viscosity of the dispersed phase, which is due to the Newtonian nature of protein isolate solutions. Similar to our results, Żmudziński, Goik, and Ptaszek (2021) reported that the emulsions prepared with 5% faba bean protein exhibited higher viscosity and stability than those with 1% protein. However, FB'-based PPCs showed lower viscosity than their counterparts. This may be due to two factors, namely, particle size and solubility of the FB' dispersions.

Firstly, as the particle size increases, there is prevention of the free flow of emulsion droplets leading to an increase in the emulsion viscosity (AZO Materials, 2015). As seen in our study's results, the droplet diameter is greater for FB-based PPCs than that of FB' (Table 1). Secondly, an increase in protein solubility makes the emulsion flow easier

Table 1
Particle size distribution parameters ($D_{4,3}$, $D_{3,2}$, span) and viscosity at $50s^{-1}$ (η_{50}) for the prepared Pickering creams.

Samples	$D_{4,3}$ (μm)	$D_{3,2}$ (μm)	d (0.9)	Span	Viscosity at $50s^{-1}$ (η_{50}) (Pa.s)
Control OF samples					
OF	32.81 ± 2.34^{a}	14.35 ± 1.68^{a}	51.71 ± 1.98^{a}	2.12 ± 0.35^{a}	0.04 ± 0.01^{a}
OF'	16.48 ± 1.34^{b}	6.59 ± 0.47^{b}	21.05 ± 0.59^{b}	2.16 ± 0.09^{a}	0.06 ± 0.02^{a}
Control FB samples					
FB2	35.03 ± 2.16^{a}	12.71 ± 3.78^{a}	49.71 ± 11.57^{a}	2.11 ± 0.64^{a}	0.01 ± 0.03^{c}
FB3	9.70 ± 0.67^{d}	2.78 ± 3.07^{cd}	18.33 ± 0.85^{b}	2.13 ± 0.21^{a}	0.03 ± 0.01^{b}
FB4	14.51 ± 3.05^{c}	4.47 ± 3.50^{cd}	22.48 ± 10.04^{b}	2.99 ± 0.34^{a}	0.08 ± 0.02^{a}
FB'2	22.58 ± 0.83^{b}	9.92 ± 0.52^{ab}	40.40 ± 2.08^{a}	1.74 ± 0.14^{b}	0.01 ± 0.01^{ad}
FB'3	4.37 ± 0.55^{fe}	0.59 ± 0.08^{d}	10.14 ± 1.04^{b}	2.97 ± 0.25^{ac}	0.01 ± 0.01^{d}
FB'4	4.58 ± 0.39^{e}	0.61 ± 0.09^{d}	10.22 ± 0.96^{b}	2.70 ± 0.06^{ac}	0.01 ± 0.01^{d}
Binary systems					
OF + FB2	20.23 ± 0.66^{a}	9.59 ± 0.51^{a}	23.37 ± 0.07^{c}	1.67 ± 0.12^{d}	0.13 ± 0.02^{d}
OF + FB3	19.25 ± 0.83^{a}	8.38 ± 1.07^{a}	26.66 ± 5.56^{c}	2.15 ± 0.15^{bc}	0.56 ± 0.00^{d}
OF + FB4	20.57 ± 1.08^{a}	7.03 ± 0.59^{a}	24.86 ± 1.88^{c}	2.49 ± 0.05^{ce}	1.55 ± 0.14^{b}
OF' + FB'2	15.89 ± 1.67^{bd}	7.56 ± 0.03^{a}	18.11 ± 0.45^{cd}	1.55 ± 0.10^{d}	0.26 ± 0.07^{de}
OF' + FB'3	15.52 ± 0.72^{bcd}	7.96 ± 0.60^{a}	27.58 ± 3.98^{bc}	2.27 ± 0.18^{b}	1.20 ± 0.34^{c}
OF' + FB'4	14.00 ± 1.63^{d}	9.78 ± 3.65^{a}	34.97 ± 2.39^{a}	3.26 ± 0.08^{a}	2.34 ± 0.16^{a}

Values are represented as means with standard deviation. Different superscripts within a column of each group set denote significant differences ($P < 0.05$) according to Tukey's pairwise comparison.

under shear which lowers the viscosity. As seen in Fig. 3a. II, the FB'-based dispersions showed more solubility than their counterparts at pH 7.0. Similar results were obtained as the physicochemical characteristics of walnut protein isolate were studied (Shi et al., 2018). In both the controls, PPCs (OF-based and FB-based), there were no significant changes observed at the $50s^{-1}$ shear rate.

Overall, for the binary components-based Pickering creams (Fig. 5c), the apparent viscosity was more than the individual controls. In addition, as the protein concentration increased, the apparent viscosity at $0.01s^{-1}$ significantly increased from 62 Pa.s (OF + FB2), 304.20 Pa.s (OF + FB3), to 1778.00 Pa.s (OF + FB4). With a greater number of droplets in the continuous phase, the movement of the droplets becomes slower, thus increasing the viscosity of the emulsion. Similar results were also supported by confocal microscopy images (Fig. 6a–f) wherein the crowding effect can be clearly observed as the protein content increases (Fig. 7c). Moreover, OF + FB4 showed significantly more value of η_{50} than its lower concentrations (Table 1). In the case of binary systems with altered pH, the viscosity tends to be significantly more than their counterparts at low shear rates. With a marginal increase in protein solubility (Fig. 3a.III), a decrease in particle size (Table 1) and more uniform droplets (Fig. 6d–c) contributed to this factor. Additionally, there was a significant increase in the viscosity at $50s^{-1}$ as the protein concentration increased with values increasing from 0.26 Pa.s (OF'+FB'2), 1.20 Pa.s (OF'+FB'3) to 2.34 Pa.s (OF'+FB'4). Overall, it can be inferred that the higher faba protein concentration (both in the case of neutral and altered pH environmental conditions), the emulsions' stability enhanced with better rheological properties. This may be due to the high concentration of hydrophobic components of the protein chains at the oil-water interface (Żmudziński et al., 2021).

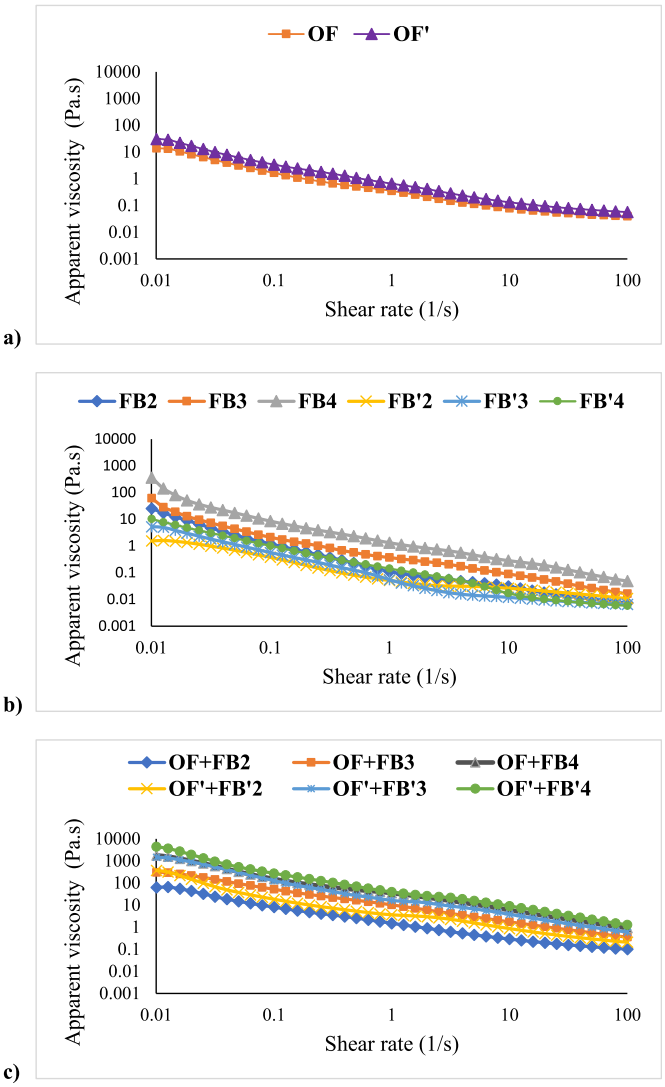

Fig. 5. Flow behaviour plots of PPCs prepared with (a) oat flour, (b) faba bean protein, and (c) binary components of oat flour and faba bean protein.

3.2.3. *Microstructure using confocal microscopy*

Visualisation of the emulsions with the help of confocal microscopy is of immense importance for a better understanding of the microstructure and the stabilising mechanism of the Pickering creams. It helps study the distribution, dimensions of the droplets and the factors influencing emulsion stability (Hu, Ting, Hu, & Hsieh, 2017). The microstructural properties of the PPCs formulated with the mixture of FB and OF are presented in Fig. 6a–f, and the ones with OF and FB, individually are presented in Fig. 6g and h (for OF) and Fig. 6i–n (for FB).

The emulsions prepared with the binary components (OF and FB), and the starch and protein phases were dyed with FITC and Rhodamine B, respectively. As seen in Fig. 6a–f, the proteins (indicated in green) surround the oil droplets (globular shaped) providing a dense barrier around the droplets to prevent coalescence. Moreover, the starch particles (indicated in blue) being the major carbohydrate in oat flour contributed towards the emulsion stabilisation due to continuous phase viscosity enhancement (Moll, Salminen, Seitz, Schmitt, & Weiss, 2022). The confocal microscopy proved to be insufficient for definitively distinguishing the protein sources of faba bean and oats in this study. With an increase in protein concentration, the average droplet diameter of the PPC droplets gradually decreased, and the distribution became denser and more uniform. This trend of a decrease in droplet size with an

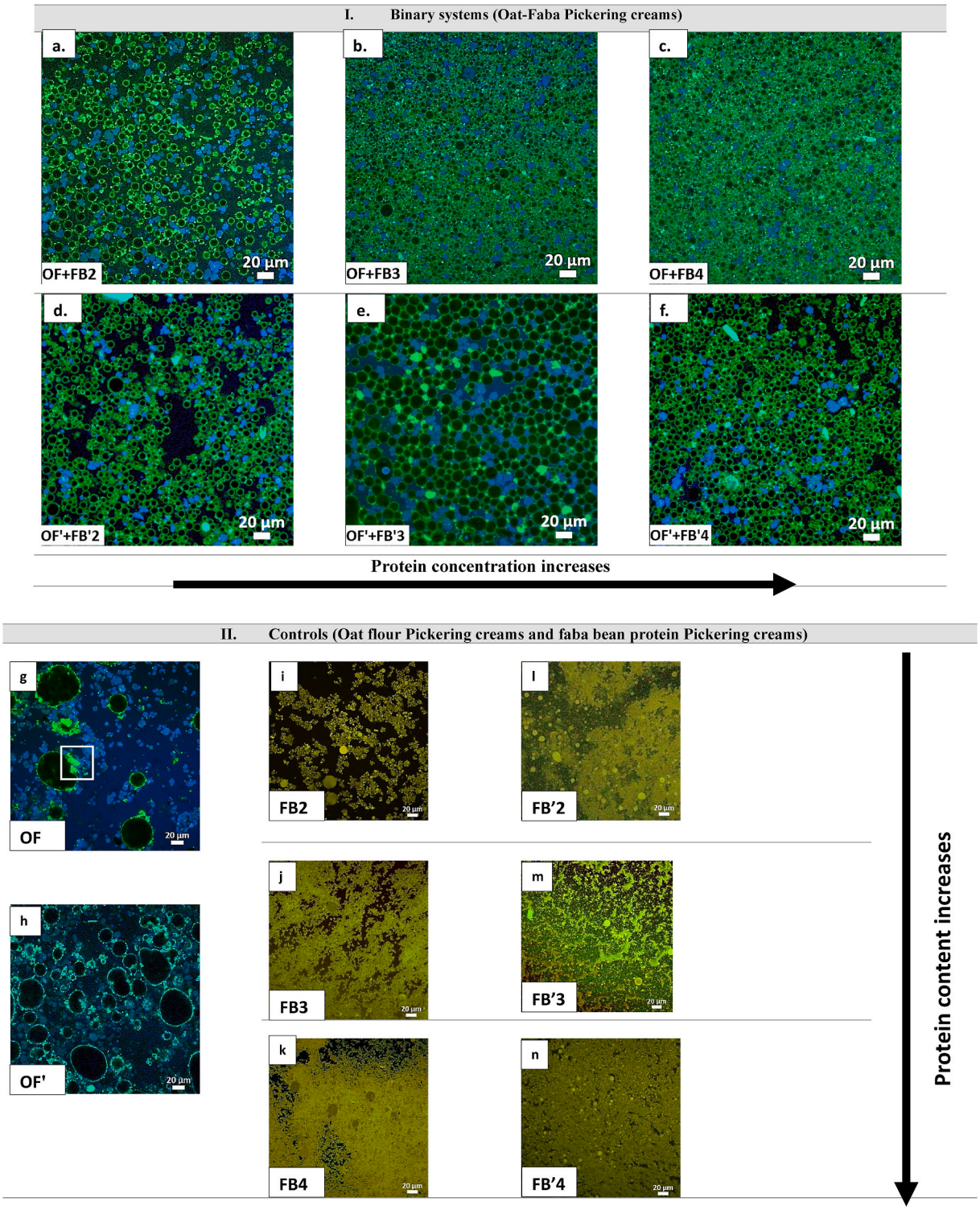

Fig. 6. Confocal microscope images of Pickering cream emulsions prepared using Controls, oat flour (g, h), faba bean protein (i–n) and the binary system (a–f).

increase in the protein concentration was supported by the particle size distribution data (Table 1) and an increase in viscosity was seen with the flow behaviour curves (Fig. 5c) Similar results were reported by Zheng, Zhao, Yi, Zhou, and Cai (2022) on the stability of PEs prepared with chestnut starch nanocrystals and macadamia protein isolate. The microstructural images of the OF- stabilised Pickering creams revealed that at neutral pH, the starch granules had a dominating factor in stabilising the emulsions (blue-coloured granules, Fig. 6g). Moreover, the formation of protein aggregates can be observed (squared area) at the interface. This could possibly be due to the internal heat generated during the homogenisation process leading to protein agglomeration (Ningtyas, Bhandari, & Prakash, 2021). However, at basic pH conditions (pH 10.0), smaller and more uniform droplet sizes were observed (Fig. 6h). Due to an increase in soluble protein content, the proteins and starch were well-homogenised and improved the emulsion stability. This phenomenon is well in agreement with the particle size distribution and emulsion stability data, wherein the $D_{4,3}$ significantly decreased in the case of OF' (16.48 µm) from OF (32.81 µm) (Table 1). This can also be

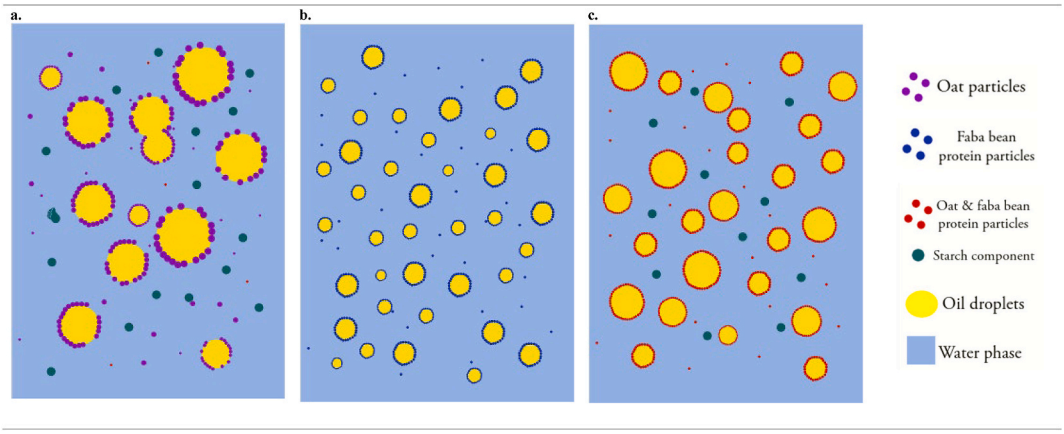

Fig. 7. Schematic representation providing an overview of emulsions stabilised with Controls oat flour (a) faba bean protein (b) and (c) binary system to understand the Pickering mechanism.

correlated to the improvement in protein solubility, emulsifying activity, and stability index of the OF' dispersions compared to OF (Fig. 3).

On studying the emulsions stabilised solely with FB isolate, the samples were dyed with Nile Red (for fat globules) and Rhodamine B (for protein particles) wherein they appeared in red and green (or yellowish), respectively. A clear and uniform distribution of fat globules embedded in the protein network was observed for all the samples. As the protein content increased from FB2 to FB4 (Fig. 6 i, j, k), the protein network became denser, and the emulsion droplets were more uniform. Moreover, the increase in protein concentration caused an increase in droplet flocculation that may be due to the possible interactions between the adsorbed proteins on the interface of the oil droplets leading to large clusters. A previous study reported that the stability and structure of the emulsions majorly depend on the adsorption and reorganisation of proteins at the interface, thus inducing various degrees of droplet formation (Lam & Nickerson, 2013). The emulsions prepared with FB at pH 3.0 (Fig. 6 l, m, n) were smaller in droplet size than their counterpart at pH 7.0. With better emulsifying properties at pH 3.0, it becomes easier for FB proteins to enter the oil-water interface. Hence, the emulsion droplets were well-homogenous, making the oil and protein droplets more tightly combined hence, enhancing the stability of the emulsion (Chen et al., 2019). This was supported by a significant improvement in the emulsifying properties and protein solubility of FB at pH 3.0 compared to neutral pH (Fig. 3).

Thus, in binary system PPCs, combining two components as stabilisers at different pH results in higher stability of emulsions due to their excellent characteristics at individual specific pH conditions. Nonetheless, when both the components stabilise the emulsions at neutral pH conditions, they exhibit good stability too. Another interesting aspect is that the dairy-based thickened cream consists of approximately 36.8% fat and 2.4% protein. With such exceptional functional characteristics, it can be said that oat flour-faba bean protein Pickering creams with 40% fat and 4% protein can be an excellent alternative to dairy-based products. Furthermore, without the use of any additional food surfactants, these binary components of Pickering emulsions offer sustainable solutions.

3.2.4. *Mechanistic aspects*

Proteins that show good solubility in the continuous phase tend to better emulsify the emulsions as they can easily migrate to the oil/water interface during emulsification, consequently leading to the formation of smaller droplets (Karaca, Low, & Nickerson, 2011). Fig. 7(a–c) show a schematic representation of the mechanisms followed by the controls, oat flour (a) and faba bean protein (b) and binary system of particles (c).

An interesting difference in the stabilising mechanism is noted on comparing the controls' PPCs. In the case of OF-stabilised PPCs (Fig. 6g and h), the starch granules dominated in stabilising the emulsion by providing a dense continuous phase with the support of proteins at the interface (Fig. 7a) whereas in the FB-stabilised PPCs (Fig. 6i–n), proteins formed a good barrier and a dense network between the oil droplets, thus preventing its destabilisation (Fig. 7b). Thus, this further confirmed that for OF-PPC, the starch component of oat dispersed media very well in water, whereas the faba bean protein particles emulsify the oil, thereby producing small emulsion droplets. Together, these two components form a complex binary arrangement by proteins emulsifying the oil and starch acting as a viscosity enhancer in the continuous phase, thus making the resulting OF + FB PPC ultra-stable, rich in viscosity and having uniformly distributed droplets (Fig. 7c).

Additionally, the interfacial activity of prepared dispersions (OF, FB and OF + FB) was studied by monitoring their ability to reduce the interfacial tension at the oil/water interfaces (Karbaschi et al., 2014). Fig. 4d shows the interfacial reduction profile over time for oat flour (OF), faba bean protein (FB) and the mixture of the particles (OF + FB). Three distinct phases were observed for all the systems, showing the typical behaviour of amphiphilic particles, as observed in Sridharan, Meinders, Bitter, and Nikiforidis (2020). The interfacial tension decreased from FB (5.13 mN/m), OF (3.7 mN/m) to OF + FB (2.56 mN/m). At the end of the kinetics, the interfacial tension for the mixture of binary particles was the lowest (0.12 mN/m), signifying better thermodynamic emulsion stability and smaller average droplet size than the control systems as supported by the droplet size data (Table 1). For all the systems, a general understanding of the adsorption kinetics can be followed. For the first step of kinetics, the protein molecules rapidly diffuse to the interface and rapidly adsorb to the interface, as seen by a rapid decrease in interfacial tension. The second step corresponds to the continuous rearrangement of particles that is accompanied by a decrease in the interfacial tension since a greater number of particles are interacting with the interface. Finally, there is a very low decrease in interfacial tension which may be due to the formation of multilayers (Ducel, Richard, Popineau, & Boury, 2004; Krägel et al., 2003). Overall, the binary mixture of particles affected both the factors of the interfacial arrangement and layer thickness at the interface, which are crucial to the stability and functional properties of the emulsions (Qin, Gao, & Luo, 2021).

3.2.5. *Storage stability*

One of the most common mechanisms for emulsion instability is creaming which takes place due to the density difference between the oil and water phases (McClements, 2009). The emulsions' ability to resist the creaming instability is dependent on several factors, such as density difference between the dispersed and continuous phase, droplet size and rheological properties (McClements, 2007). Fig. 8a and b present the

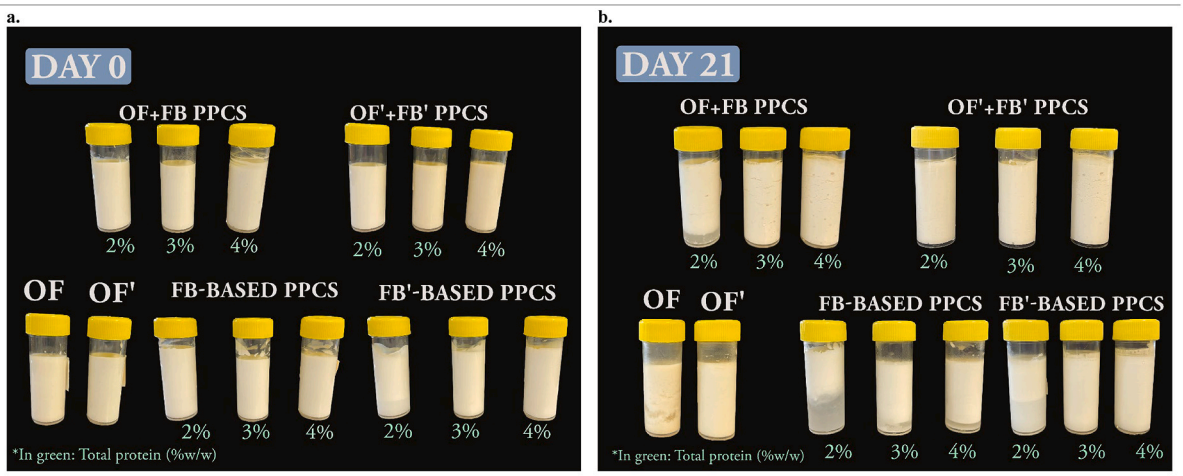

Fig. 8. Storage stability images of PPCs captured on (a) day 0 and (b) day 21 of preparation.

images that were captured on day 0 and day 21 after the preparation of the PPCs. Table 2 presents the creaming index determined after 21 days of storage.

In the case of PPCs stabilised with oat flour, OF' showed no destabilisation due to creaming, however, at neutral conditions the emulsion showed a creaming index of 22.22%. This behaviour of OF'-PPC can be attributed to the improvement in emulsifying properties of the protein at alkaline pH that emulsified the oil droplets better and resisted any changes (Fig. 3b) In the case of FB-stabilised emulsions at neutral conditions, phase separation was observed in all the samples with CI decreasing from 47.27% (FB2), 17.39% (FB3) to 10.00% (FB4) as the protein concentration increased. According to previous studies, rapid separation occurs in emulsions wherein the solid particles are unable to cover the oil-water interface thoroughly, thus resulting in a complete mobile continuous phase (Pal, 2019). The PPCs prepared with FB' showed improved stability in the case of FB'3 and FB'4 with no phase separation after 21 days of storage. This was attributed to a significant increase in their protein solubility and emulsifying properties than their counterparts at pH 7.0 (Fig. 3a. II, Fig. 3b).

In the case of PPCs prepared with binary components, no phase separation was observed even after 21 days except in the case of OF'+FB'2 (CI: 10.50%) which may be due to large emulsion droplet sizes (Table 1) that rapidly coalesced together. This phenomenon showed how the emulsions prepared with these two components together, specifically at altered pH conditions, exhibited such ultra stability that there were no visual signs of flocculation or coalescence between the emulsion droplets. Moreover, after 21 days of storage, when the samples were gently stirred, no oil separation was observed at the top and the samples appeared just as day 0 (images not shown).

Table 2
Creaming index of the Pickering creams determined on day 21 after the preparation of the PPCs.

Control OF samples		Control FB samples		Binary systems	
Emulsions	CI (%)	Emulsions	CI (%)	Emulsions	CI (%)
OF	22.25 ± 0.50^{a}	**FB2**	47.27 ± 2.05^{a}	**OF + FB2**	10.50 ± 2.00^{a}
OF'	0.1 ± 0.01^{b}	**FB3**	17.39 ± 2.61^{c}	**OF + FB3**	0.10 ± 0.01^{b}
		FB4	10.00 ± 0.80^{d}	**OF + FB4**	0.10 ± 0.01^{b}
		FB'2	38.56 ± 1.15^{b}	**OF' + FB'2**	0.10 ± 0.01^{b}
		FB'3	0.1 ± 0.01^{e}	**OF' + FB'3**	0.10 ± 0.01^{b}
		FB'4	0.1 ± 0.01^{e}	**OF' + FB'4**	0.10 ± 0.01^{b}

Values are represented as means with standard deviation. Different superscripts within a column denote significant differences ($P < 0.05$) according to Tukey's pairwise comparison.

4. Conclusion

This study demonstrated the utilisation of a binary system of oat flour and faba bean protein particles as stabilisers to fabricate plant-based Pickering creams with minimal processing and no use of external surfactants. The creams with greater functionality and higher stability were achieved by enabling solubility and access to functional groups/charges of proteins in both components through a mild pH shift treatment. The fabricated PPCs containing 3–4% protein exhibited excellent functional characteristics, including small and uniformly distributed emulsion droplets with a tuneable viscosity range. This approach has also afforded good storage stability (no phase separation over 21 days) of storage at 4 °C. The microstructural analysis gave an insight into the Pickering stabilising mechanism that showed the binary role of these systems by using a dispersed starch component of oat as a viscosity modifier and protein components as stabilisers. Hence, these findings can be of great importance to the sustainable engineer of plant-based Pickering emulsion systems and cream products with minimal processing methods. This study can also have implications for future studies investigating the behaviour of oat flour with other plant proteins (soy, pea, rice) in a multi-component system and how other (enzymatic and thermal) treatments will influence the preparation of plant-based creams with desired functionalities.

CRediT authorship contribution statement

Kirti Rawal: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Pratheep Kumar Annamalai:** Writing – review & editing, Supervision, Conceptualization. **Bhesh Bhandari:** Writing – review & editing, Supervision. **Sangeeta Prakash:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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