

# Emulsification properties of plant and milk protein concentrate blends

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## Abstract

Blending is a promising strategy during the partial replacement of plant with animal proteins. This, however, may lead to alteration in the technofunctional properties of the resultant blends. In this study, soy, rice and pea protein concentrates (SPC, RPC and PPC, respectively) were blended with milk protein concentrate (MPC) at different ratios: 25:75, 50:50 and 25:75 and the technofunctional properties relevant to their emulsification behaviour, e.g., emulsion stability, viscosity and water and oil binding capacity, were investigated. At equivalent concentrations, the plant protein concentrates had higher apparent viscosities compared to MPC and the blends. RPC-MPC, at all ratios, had a lower oil binding capacity when compared with the SPC-MPC and PPC-MPC blends. Plant protein-MPC blends showed higher emulsion stability compared to the individual plant protein concentrates. Blending MPC with plant protein concentrates resulted in promising improvements in emulsification behaviour of relevance to different composite protein ingredient applications.

**Emulsification properties of plant and milk protein concentrate blends** Mohammadreza Khalesi<sup>a,b</sup>, Shauna Dowling<sup>a</sup>, Jack Comerford<sup>a</sup>, Ciara Sweeney, Richard J. FitzGerald<sup>a\*,a</sup> *Department of Biological Sciences, University of Limerick, Ireland<sup>b</sup> School of Agriculture and Food Science, University College Dublin, Ireland\** Correspondence to: R.J. FitzGerald, Department of Biological Sciences, University of Limerick, Ireland; E-mail address: dick.fitzgerald@ul.ie **Abstract:** Blending is a promising strategy during the partial replacement of plant with animal proteins. This, however, may lead to alteration in the technofunctional properties of the resultant blends. In this study, soy, rice and pea protein concentrates (SPC, RPC and PPC, respectively) were blended with milk protein concentrate (MPC) at different ratios: 25:75, 50:50 and 25:75 and the technofunctional properties relevant to their emulsification behaviour, e.g., emulsion stability, viscosity and water and oil binding capacity, were investigated. At equivalent concentrations, the plant protein concentrates had higher apparent viscosities compared to MPC and the blends. RPC-MPC, at all ratios, had a lower oil binding capacity when compared with the SPC-MPC and PPC-MPC blends. Plant protein-MPC blends showed higher emulsion stability compared to the individual plant protein concentrates. Blending MPC with plant protein concentrates resulted in promising improvements in emulsification behaviour of relevance to different composite protein ingredient applications. **Keywords:** Plant protein; Milk protein concentrate; Blend; Emulsion properties.

## Introduction

The global population is progressively increasing leading to growing demand for dietary protein (United Nations, 2019). Currently, the proteins used to meet these demands mainly originate from animal sources, e.g., meat and dairy proteins. Milk protein concentrates (MPC) are dairy ingredients containing 42-85% protein (Khalesi and FitzGerald, 2022a). MPCs are used in products such as in dietary supplements,

nutrition bars and sports beverages due to their high level of essential amino acids (EAAs) along with their low lactose content, particularly in the case of higher protein content MPCs. While animal proteins have numerous advantages in food product applications, the latest developments in food sustainability focus on the impact of animal origin products, particularly in terms of their climate change and economic implications. Accordingly, the demand for alternative protein sources, mainly from plants, is increasing. Many plant proteins have good functional (e.g., emulsification) properties in food applications which enables them to serve as potential substitutes for animal origin proteins. Nevertheless, there are some issues which may restrict the widespread application of plant proteins in food products. For instance, some plant proteins contain low levels of certain EAAs and high levels of anti-nutritional factors (Foegeding and Davis, 2013). In addition, some plant proteins display relatively poor technofunctional properties (Nikbakht Nasrabadi, et al., 2021). Therefore, there is an increased interest on the functionality and nutritional quality of the hybrid protein products, i.e., combinations of plant with animal proteins (Reidy et al., 2013; Khalesi and FitzGerald, 2021a). Emerging evidence suggests that blending plant with animal proteins could increase the utilization of plant proteins while improving their overall functionality and nutritional quality. Blending can also be considered as a new marketing opportunity for food manufacturers to develop products with novel characteristics (such as improved technofunctionality and sensory properties). Blending of pea protein isolate (PPI) and whey protein isolate (WPI) improved the functionality of PPI in a mixed protein system formulation (Kristensen et al., 2021). It has also been shown that blending of skim milk powder with pea protein concentrate (PPC) modified the technofunctional properties of first age infant formula (Le Roux et al., 2020). It increased the viscosity and reduced the solubility, while it did not change the emulsion stability (ES) of the product. Ho et al., (2018) reported that plant-derived emulsifiers generated with soy protein isolate (SPI) and PPI were suitable replacements for dairy proteins including WPI and sodium caseinate. It has been reported that milk protein-soy protein (SP) blends have higher apparent viscosity ( $\eta_{app}$ ) compared to micellar CN (Beliciu et al., 2013). Alves and Tavares (2019) stated that the partial replacement of animal protein with plant protein is the first step toward the reduction of the environmental impacts associated with animal food consumption. However, it is still relatively unknown how plant and dairy proteins may behave when in blends. Limited knowledge appears to exist on the impact of blending of dairy and plant proteins on the functionality, e.g., emulsification properties, in different product. Therefore, acquisition of this knowledge may help in the targeted design of balanced blends for different functional and nutritional applications. The hypothesis is that blending plant with animal proteins has the potential to yield protein mixtures with novel emulsion properties due to the potential for interactive effects between the different origin proteins. This in turn may lead to the development of new functionality and ingredient applications. The objective of this study was to evaluate properties relevant to the emulsification behaviour of blends created using different plant proteins, i.e., soy (SPC), pea (PPC) and rice protein concentrate (RPC) with MPC at different ratios.

## Material and methods

### *Materials*

SPC, PPC and RPC from Pulsin Ltd. (Gloucester, UK) were obtained at a local healthfood store and MPC85 (85% (w/w) protein) was obtained from a commercial manufacturer. Corn oil was purchased from a local food market. Sodium hydroxide (NaOH) and acetic acid were from Fisher Scientific (Dublin, Ireland). Kjeldahl catalyst tablets, sulphuric acid (> 98%), boric acid, 2-mercaptoethanol, methanol, protein molecular mass markers (6.5-200.0 kDa) and Sudan III were from Sigma-Aldrich (Dublin, Ireland). Hexane was from Honeywell International Inc. (Dublin, Ireland). Coomassie R, Laemmli buffer, Mini-Protean TGX 4-20% pre-cast polyacrylamide gels were from Bio-Rad Laboratories Inc. (CA, USA) and sodium dodecyl sulfate (SDS) was from National Diagnostics (GA, USA).

### *Proximate analysis and pH determination*

Moisture, ash, lipid and protein contents were determined according to Khalesi and FitzGerald (2021a).

1. *Blending of plant protein samples with MPC* Different blends having different ratio of proteins from

plant sources and MPC85 were generated as schematically outlined in Figure 1.

2. *Technofunctional property analysis*
3. *Emulsification*

Freeze-dried samples of each individual plant protein concentrate, MPC and the plant protein-MPC blends were resuspended with dH<sub>2</sub>O and adjusted to pH 7.0 to give a 0.025% (w/v) protein suspension. Sudan Red III (40 mg) was added to 1 L of corn oil, after which 6 g was added to 14 g of each protein sample suspension. Samples were then homogenised using an Ultra-Turrax (IKA T25, Staufen, Germany) for 1 min at 16000 rpm in order to create an emulsion. Immediately after homogenisation, an aliquot of sample (18 µL) was 100 fold diluted with 0.1% (w/v) SDS to reach a volume of 1.8 mL. The absorbance ( $A$ ,  $\lambda_{500}$ ) of the bottom half of the emulsion sample was measured ( $n = 3$ ) using a UV-Vis 1800 spectrophotometer (Shimadzu, Canby, USA) at T0 and 30 min (T30) after emulsion formation. ES was determined according to eq 1:  $ES (\%) = \frac{A_{T30}}{A_{T0}} \times 100 (1)$

where  $A_{T30}$  and  $A_{T0}$  represent the absorbance ( $\lambda_{500}$ ) at T30 and T0 (min), respectively.

#### *Apparent viscosity ( $\eta_{app}$ )*

An aliquot (16 mL) of each suspension equivalent to 5% (w/v) protein prepared after reconstitution of the freeze dried samples was analysed using a Brookfield DV-II viscometer (Analytica, Dublin, Ireland) at 30°C ( $n = 3$ ) at a shear rate of 6 s<sup>-1</sup> for the PPC, SPC and their MPC blends and at 100 s<sup>-1</sup> for the RPC containing samples. The  $\eta_{app}$  of MPC was measured at both share rates (i.e., 6 and 100 s<sup>-1</sup>).

#### *Water holding capacity (WHC) and oil binding capacity (OBC)*

The WBC and OBC for the different freeze-dried blends was determined ( $n = 3$ ) by resuspension of each sample/blend in dH<sub>2</sub>O or corn oil to reach a final concentration of 5% (w/v) on a protein basis, vortexing for 30 s followed by centrifugation (320R Hettich centrifuge, Tuttlingen, Germany) at 5000  $g$  for 30 min. The WHC and OBC were calculated according to Khalesi and FitzGerald (2022b).

#### *Solubility*

The overall solubility was determined on the basis of the total solids (TS) of the protein concentrate/blend samples and the particle size (PS) of aqueous protein suspensions (Khalesi and FitzGerald, 2021b).

#### *Statistical analysis*

Data values were presented as mean  $\pm$  standard deviation (SD). One-way analysis of variance (ANOVA) followed by the *Tukey post hoc* comparison test was carried out to test for significant differences using Minitab® Release 15 for Windows. A  $p$  value  $< 0.05$  was considered as statistically significant.

## **Results and discussion**

### *Proximate analysis and sample properties*

As shown in Table 1, it is evident that there was some variation in the moisture, protein, ash and the lipid contents of the different test samples. The moisture content in the samples ranged from 1.64-5.58% with the lowest value being for RPC and the highest value for SPC. MPC had the highest overall protein content (84.17 $\pm$ 0.79%), while among the plant protein concentrates, SPC had the highest protein content (81.11 $\pm$ 0.77%). The protein content in PPC was 71.01 $\pm$ 0.25%. The lipid content in MPC85 (1.31 $\pm$ 0.07%) was the lowest among the samples as it is manufactured from skim milk. Significant differences were found between the lipid content in each of the plant protein samples. The lipid content in RPC (9.70 $\pm$ 0.37%) and PPC (8.13 $\pm$ 0.17%) was higher than in SPC (1.79 $\pm$ 0.11%). The ash content showed less variance, the highest mean value being 6.96% in MPC and the lowest being 5.47% for SPC. PPC had the highest mean ash level (6.48%) among the plant protein samples. The reconstitution pH values ranged from pH 6-8, with RPC being slightly acidic (pH 6.09) and PPC being slightly basic (pH 8.00). The mean pH of SPC was similar to MPC (7.15 vs 7.09).

### Technofunctional properties.

*Αππαρεντ ισχοσιπ* ( $\eta_{app}$ ): The  $\eta_{app}$  varied across the plant blends (Figure 2). Overall, SPC and PPC produced the most viscous suspensions. The  $\eta_{app}$  for SPC ( $64.3 \pm 10.9$  mPa.s) and PPC ( $62.5 \pm 8.7$  mPa.s) were not significantly different ( $p > 0.05$ ). Both 100% SPC and PPC gave a higher viscosity value than MPC ( $36.8 \pm 1.2$  mPa.s) at a similar shear rate. Previously, the  $\eta_{app}$  of SPC was reported to be similar to sodium caseinate while it was two times higher when compared to WPI (Webb et al., 2002). The results herein demonstrated that PPC and SPC have, under certain circumstances, elevated viscosity properties which may be desirable for emulsions as well as in the formulation of high viscosity requiring products, e.g., plant-based yoghurts and ice cream. The  $\eta_{app}$  of RPC ( $1.4 \pm 0.1$  mPa.s) was not significantly ( $p > 0.05$ ) different from MPC ( $1.5 \pm 0.1$  mPa.s) when tested at a similar shear rate (i.e.,  $6 \text{ s}^{-1}$ ). The mean viscosity values of the SPC-MPC blends tended to increase as the proportion of SPC in the blends increased. This is associated with the higher  $\eta_{app}$  of SPC compared to MPC. The  $\eta_{app}$  associated with the blends generated with PPC was higher ( $p < 0.05$ ) than those generated with SPC, except when at a ratio of PPC-MPC of 50:50 (Figure 2a). A previous report showed that the inclusion of PPC in infant formula produced with skim milk (50:50) enhanced the viscosity while the inclusion of faba bean protein at the same ratio did not change the overall viscosity (Nadathur et al., 2017). The  $\eta_{app}$  of WPI was reported to be significantly increased on blending with PPI (Tarrega et al., 2012). The  $\eta_{app}$  of sodium caseinate was reported to be higher than PPI and a PPI-sodium caseinate hydrid blend (Yerramilli et al., 2017). The reconstituted suspensions of the RPC-MPC blends had the lowest  $\eta_{app}$  among the plant-MPC blends (Figure 2b). Increasing the quantity of RPC did not increase the  $\eta_{app}$ , with the RPC-MPC 25:75 blend yielding the highest  $\eta_{app}$  value ( $3.76 \pm 0.13$  mPa.s) among the RPC-MPC blends ( $p < 0.05$ ). The results showed that the hybrid blends created with RPC and MPC had a higher  $\eta_{app}$  compared to MPC and RPC alone, indicating possible interactions between the RPC and MPC protein suspensions.

Overall, the PPC-MPC and SPC-MPC blends may prove useful for high viscosity requiring applications. It should also be noted that the presence of other non-proteinaceous components may cause differences between the  $\eta_{app}$  of various plant protein ingredients. In addition, most of the blend samples gave  $\eta_{app}$  values higher than that for MPC alone suggesting the possibility of replacement of MPC with plant proteins for high viscosity requiring purposes. In general, higher viscosity is associated with better emulsification properties (Dapčević-Hadnadev et al., 2019).

### WHC and OBC

MPC had a higher ( $p < 0.05$ ) WHC ( $349 \pm 24$  g water/100 g protein) compared to the individual plant protein concentrates tested (Figure 3). The extensive interaction between protein components, especially the CNs, in MPC and water molecules is considered as the main reason for the high WHC of MPC. In addition, the plant protein concentrates had a higher lipid content, thus expectedly; they showed a lower affinity to retain water compared to MPC. SPC had a higher ( $p < 0.05$ ) WHC ( $184 \pm 8$  g water/100 g protein) than RPC ( $26 \pm 2$  g water/100 g protein) and PPC ( $129 \pm 5$  g water/100 g protein). Some specific protein components of SPC (particularly the 11S globulin) have previously been shown to contribute to the WHC and to the formation of stable protein gels (Onwulata et al., 2014). The higher WHC of SPC compared to PPC and RPC may also be associated with the lower lipid content of the SPC ingredient studied herein. Among the blended samples, SPC-MPC 25:75 ( $394 \pm 9$  g water/100 g protein) had the highest ( $p < 0.05$ ) WHC. As the proportion of SPC increased and MPC decreased in the SPC-MPC blends, the WHC decreased. All SPC-MPC blends had a notably higher WHC in comparison to the RPC-MPC and PPC-MPC blends. Minimal variation was observed in the WHC between any of the RPC-MPC blends where the RPC-MPC blends had the lowest WHC ( $23\text{--}33$  g water/100 g protein). The WHC of the PPC-MPC blends was significantly lower ( $p < 0.05$ ) than the MPC sample. The WHC of the PPC-MPC 25:75 blend ( $129 \pm 5$  g water/100 g protein) was higher than 100% PPC ( $94 \pm 2$  g water/100 g protein) ( $p < 0.05$ ). These results indicate interactions between SPC-MPC (25:75) resulting in an improvement in the WHC. This may be beneficial for the partial replacement of MPC for applications where high WHC and gelation properties are required, e.g., in yoghurt and cheese type products. Variability in the results of OBC of individual plant proteins was observed which may be

associated with the composition of the plant proteins, especially the lipid and protein contents and also the surface located composition of the powder particles. As shown in Figure 3, no plant protein concentrate or plant protein-MPC blend reached an OBC similar to that of MPC (239 g oil/g protein). This may be related to the presence of high levels of surface lipid in MPC85 which has been shown to consist of > 15% of its surface composition (Chew et al., 2014). In addition, the low OBC of plant proteins has previously been reported to be due to a large proportion of hydrophilic protein groups on their surfaces (Chavana et al., 2001). The OBC of SPC ( $180 \pm 8$  g oil/100 g protein) and SPC-MPC blends (ranging between 66-123 g oil/100 g protein) was highest amongst the plant protein concentrates and plant protein-MPC blends. RPC and RPC-MPC blends performed the poorest across all plant protein concentrates and plant protein-MPC blends in terms of OBC. Among the RPC-MPC blends, RPC-MPC 25:75 yielded the highest OBC at  $51 \pm 2$  g oil/100 g protein, while the lowest OBC ( $33 \pm 2$  g oil/100 g protein) was seen for RPC-MPC 50:50. The OBC of PPC was  $89 \pm 3$  g oil/100 g protein, which is in the range of previous reports showing that the OBC of commercial PPC was  $\sim 100$  g oil/100 g protein. The ratio of 11S to 7S globulins was suggested to have an impact on the OBC of PPI (Reinkensmeier et al., 2015). Among PPC-MPC blends, PPC-MPC 25:75 yielded the highest OBC at  $96 \pm 4$  g oil/100 g protein, while the lowest OBC ( $82 \pm 1$  g oil/100 g protein) was seen for PPC-MPC 75:25. These results showed that the blending of plant with milk proteins did not increase the OBC of the samples. The higher OBCs in the SPC and PPC blends (in comparison with the RPC blends) may be an advantage for some functionalities such as for emulsification and applications related to the formulation of breads, cakes and muffins.

### *Solubility (%)*

As shown in Figure 4, the lowest solubility of the plant protein concentrates tested was associated with SPC ( $43.70 \pm 2.33\%$ ) and PPC ( $55.74 \pm 2.75\%$ ), while the highest was associated with RPC ( $80.94 \pm 0.35\%$ ). The solubility varied between the different blends within each plant protein sample highlighting the differences in their interactions with MPC (Figure 4). The mean solubility of the RPC-MPC blends was higher than both the SPC- and PPC-MPC blends. Overall, the RPC-MPC 75:25 blend gave the highest solubility ( $86.89 \pm 1.70\%$ ) among the blends ( $p < 0.05$ ). The solubility of PPC was lower than RPC ( $p < 0.05$ ). However, blending PPC with MPC increased its solubility, with the highest being associated with PPC-MPC 50:50 and 25:75. A higher proportion of PPC, however, reduced solubility. SPC had the poorest solubility among the individual plant proteins. Blending SPC-MPC enhanced the overall solubility of SPC. Among the SPC-MPC blends, the highest solubilisation was related to the SPC-MPC 25:75 blend. The enhanced solubility of some plant protein-MPC blends was evidence for a synergistic relationship between plant proteins and MPC and their interactions with the aqueous phase. In addition, the particle size distributions of 5% (w/v, protein) of the aqueous powder suspensions of each plant protein concentrate and the blends were measured (Section 2.4.4) using laser light scattering (Table 2). The Sauter mean diameter  $D[3,2]$  of MPC was  $38.55 \pm 2.11$   $\mu\text{m}$  which was lower than for SPC and PPC, while it was larger than for the RPC sample. The  $D[3,2]$  for all tested samples ranged between 8.01 and 101.79  $\mu\text{m}$ , with the RPC-MPC blends having the lowest  $D[3,2]$  on average (ranging between 8.01 and 23.33  $\mu\text{m}$ ). The low  $D[3,2]$  values for the RPC-MPC blends was in line with their higher solubility and lower  $\eta_{\text{app}}$  in comparison to PPC- and SPC-MPC blends. However, the polymodal particle size distribution seen in the RPC samples (Figure 5) suggests that these suspensions may not remain stable over time, as those particles may further coalesce. This polydispersity was not observed in either the PPC or the SPC samples. The  $D[3,2]$  associated with the SPC-MPC and PPC-MPC blends was in the range of 57.52-101.79  $\mu\text{m}$ . Among the SPC blends, the SPC-MPC 75:25 sample had the highest  $D[3,2]$  ( $88.36 \pm 1.93$   $\mu\text{m}$ ). Similarly, among the PPC blends, the PPC-MPC 75:25 blend had the highest  $D[3,2]$  ( $98.50 \pm 3.29$   $\mu\text{m}$ ). These results showed that the presence of a lower proportion of MPC in SPC-MPC and PPC-MPC blends increased the PS, which is in accordance with the lower solubility observed for these blends. The SSA of the blends was compared (Table 2). The PPC-MPC and SPC-MPC blends gave the lowest SSA. As expected, the RPC-MPC 25:75 blend, which had the lowest  $D[3,2]$ , presented the highest SSA ( $0.64$   $\text{m}^2/\text{g}$ ). In general, particles with smaller sizes and larger surface areas may be associated with positive implications for the stability of emulsified foods (Malaki Nik et al., 2009). According to these results and previous literature, the formation of soluble plant protein-MPC blends depends on the plant protein

source, the proportion of the plant protein in the blend and it also depends on the interactions between the plant proteins and CN/WP fractions in MPC.

### *Emulsion stability (ES%)*

Among samples with 100% plant protein, the lowest ES was associated with the PPC emulsion (51%). There were clear differences on the impact of various proportions of plant proteins on ES. Blends which yielded 100% stability after 30 min holding at room temperature were RPC-MPC 50:50, RPC-MPC 75:25, SPC-MPC 75:25, SPC-MPC 100:0 and PPC-MPC 50:50 (Figure 6). The RPC-MPC 25:75 (11%), SPC-MPC 25:75 (21%) and SPC-MPC 50:50 (31%) yielded the lowest ES. Incorporation of 75% plant protein to MPC yielded a high ES in all cases. Incorporation of 50% plant protein to MPC also yielded a high ES in the case of the RPC- and PPC-MPC blends. The results of ES suggest PPC had the highest extent of interaction with the milk proteins and perhaps the PPC-MPC blends had the highest interfacial energy among the blends, given that the 100% PPC sample exhibited the lowest ES. This considerably improved with the introduction of MPC and was highly stable at a ratio of 50:50 (100%). Furthermore, the PPC-MPC 25:75 emulsion showed higher ES (90%) compared to the other two plant protein samples at the same ratio. This implies a strong interfacial film being generated by the PPC-MPC blend which was in accordance with a previous report on the emulsion stability of WPI-PPI blends (50:50) (Ho et al., 2018). A synergistic interaction between pea and milk protein has also been highlighted by Hinderink et al. (2020) who observed PPI-sodium caseinate and PPI-WP emulsions which remained stable over 14 d, unlike emulsions formed by either PPI and sodium caseinate alone. The rate of adsorption of the blend of dairy proteins (WPI and sodium caseinate) and PPI at the air-water interface was higher than individual proteins showing a synergistic effect arising from blending. In addition, blends of sodium caseinate and PPI had an improved interfacial strength (which is an indication for ES) compared to sodium caseinate alone and thicker films were formed compared to all individual proteins. The emulsion activity of the blends generated with PPI and WPI was recently shown to be higher than the PPI alone (Kristensen et al., 2021). This effect may be associated with reduced flocculation and coalescence by the proteins due to electrostatic interaction between surface protein charges. In addition, Kristensen et al., (2021) found that pea proteins are capable of adsorbing to the oil-water interface after introduction to a pre-adsorbed WP interface. On the other hand, Hinderink et al., (2021) found that a pre-adsorbed PPC at the oil-water interface can be replaced with  $\beta$ -lactoglobulin. Addition of PPC to infant formula has been reported to have no effect on the emulsion characteristics of the product (Ju et al., 2006). It was also found herein that the lower proportion of SPC was less beneficial in the SPC-MPC (25:75) blend system for ES. Increasing the proportion of SPC improved ES. A major increase in ES was observed by increasing the SPC content in the SPC-MPC blend from 50 to 75%. This may be indicative of the high ES of SPC *per se*, which was previously reported by Molina et al. (2001). Ji et al., (2015) reported higher long-term ES in sodium caseinate-SPC emulsions compared to sodium caseinate or SPI stabilized emulsions. Synergic effects on the interfacial strength and viscoelastic film at the air/water interface were also reported for  $\beta$ -conglycinin (7S) and  $\beta$ -lactoglobulin (50:50) compared to the individual proteins (Pizones Ruiz-Henestrosa et al., 2014). To our knowledge, this appears to be the first report on the emulsion properties of plant protein-MPC blends. These findings showed that while various plant protein sources had different emulsion properties, the interactions of these proteins with MPC at certain ratios enhanced their ES. This is advantageous for the partial replacement of MPCs with plant proteins to yield highly stable hybrid emulsions for different applications such as in the manufacture of soups and sauces.

### **Conclusions**

The global demand for health promoting foods and the desire to reverse the impact of humans on the Earth's environment is increasing. Therefore, the goal to partially or fully replace animal-based food products with sustainable plant products and to reduce the consumption of animal products prevails. In addition, the demonstration of new functions for novel protein ingredients compared to existing highly consumed animal origin proteins is necessary in order to expand the protein market. Incorporation of plant proteins (SPC, PPC and RPC) with MPC in different ratios in this study showed that these blends may be successfully used for partial replacement of MPC. The blends significantly differed regarding their emulsification prop-

erties. Multiple blends arising from SPC-, RPC- and PPC-MPC were shown to have functional properties that may be useful in specific food applications. Among the blends, SPC-MPC 25:75, PPC-MPC 25:75 and RPC-MPC 50:50 were shown to be the most suitable in regard to their overall emulsification properties. From the findings in this study, it is clear that the proportion of each component in the blend is an important factor that can be modified during the generation of plant protein-MPC blends on the basis of the target application (e.g., as an alternative protein source during infant formula manufacture). Some of the functional properties of plant protein-MPC blends obtained in this study are promising for different applications (such as those required for high viscosity and stable emulsions) in the food industry. Different types of protein-protein interactions take place depending on the characteristics of the individual proteins in the plant protein concentrates and in MPC. Therefore, there is a need for molecular level studies to unravel the nature of these interactions as well as the potential impact of conventional/novel processing conditions on same.

**Contributions of authors** *Mohammadreza Khalesi*: Conceptualization; Formal analysis; Methodology, Writing-original draft and Funding acquisition; *Shauna Dowling*: Investigation; Formal analysis and Data curation; *Jack Comerford*: Investigation; Formal analysis and Data curation; *Ciara Sweeney*: Investigation; Formal analysis and Data curation; *Richard J. FitzGerald*: Conceptualization; Supervision; Editing and Funding acquisition

**Declaration of interest** The authors declare that there is no conflicts of interest.

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**Table of Abbreviations**

Description	Abbreviation
amino acid	AA
apparent viscosity	$\eta_{app}$
casein	CN
diafiltration	DF
emulsion stability	ES
essential amino acids	EAA
milk protein concentrate	MPC
milk protein isolate	MPI
molecular weight	MW
oil binding capacity	OBC
particle size	PS
pea protein	PP
pea protein concentrate	PPC
pea protein isolate	PPI
polyacrylamide gel electrophoresis	PAGE
rice protein	RP
rice protein concentrate	RPC
rice protein isolate	RPI
sodium dodecyl sulfate	SDS
soy protein	SP
soy protein concentrate	SPC
soy protein isolate	SPI
specific surface area	SSA
total solids	TS
ultrafiltration	UF
water holding capacity	WHC
whey protein	WP
whey protein concentrate	WPC
whey protein hydrolysate	WPH
whey protein isolate	WPI

- REFERENCES** Alves A.C., Tavares G.M. Mixing animal and plant proteins: Is this a way to improve protein techno-functionalities?. *Food Hydrocolloid*.2019, 97, 105171. Behrend O, Schubert KAH. Influence of continuous phase viscosity on emulsification by ultrasound. *Ultrason Sonochem*. 2000, 7, 77–85 Beliciu C.M. and Moraru C.I. Physio-chemical changes in heat treated micellar casein-Soy protein mixtures, *LWT Food Sci. Technol*.2013, 54(2), 469–476. Chavana U.D., McKenzie D.B., Shahidi F. Functional properties of protein isolates from beach pea (*Lathyrus maritimus* L.) 2001,74(2), 177–187. Chew J.H., Liu W., Fu N., Gengenbach T., Chen X.D., Selomulya C. Exploring the drying behaviour and particle formation of high solids milk protein concentrate. *J. Food Eng*. 2014, 143,186–194. Dapčević-Hadnadev T., Dizdar M., Pojić M., Krstonošić V., Zychowski L.M., Hadnadev M. Emulsifying properties of hemp proteins: Effect of isolation technique. *Food Hydrocolloid*. 2019, 89, 912–920. Fathi P., Moosavi-Nasab M., Mirzapour-Kouhdasht A., Khalesi M. Generation of hydrolysates from rice bran proteins using a combined ultrasonication-Alcalase hydrolysis treatment. *Food Biosci*. 2021,42, 101110. Foegeding E.A., Davis J.P. Food protein functionality: A comprehensive approach. *Food Hydrocolloid*. 2011, 25(8), 1853–1864. Hinderink E.B.A., Münch K., Sagis L., Schroën K., Berton-Carabin C.C. Synergistic stabilisation of emulsions by blends of dairy and soluble pea proteins: Contribution of the interfacial composition, *Food Hydrocolloid*. 2019, 97, 105206. Hinderink E.B.A., Sagis L., Schroën K., Berton-Carabin C.C. Behavior of plantdairy protein blends at air-water and oil-water interfaces, *Colloids Surf. B*. 2020, 192 (April), 111015. Hinderink E.B.A., Sagis L., Schroën K., Berton-Carabin C.C. Sequential adsorption and interfacial displacement in emulsions stabilized with plant-dairy protein blends. *J. Colloid Interface Sci*. 2021, 583, 704–713. Ho K.K.H.Y., Schroen K., Martín-Gonzalez M.F.S., Berton-Carabin C.C. Synergistic and antagonistic effects of plant and dairy protein blends on the physicochemical stability of lycopene-loaded emulsions. *Food Hydrocolloid*. 2018, 81, 180–190. Ji J., Zhang J., Chen J., Wang Y., Dong N., Hu C., Chen H., Li G., Pan X., Wu C. Preparation and stabilization of emulsions stabilized by mixed sodium caseinate and soy protein isolate. *Food Hydrocolloid*.2015, 51, 156–165. Ju Z.Y., Hettiarachchy N.S., Rath N. Extraction, denaturation and hydrophobic properties of rice flour proteins. *J. Food Sci*. 2006,66(2), 229–232. Khalesi M., FitzGerald R.J. *In vitro* digestibility and antioxidant activity of plant protein isolate and milk protein concentrate blends. *Catalysts*. 2021a, 11, 787. Khalesi M, FitzGerald R.J. Physicochemical properties and water interactions of milk protein concentrate with two different levels of undenatured whey protein. *Colloids Surf. A*. 2021b, 629, 127516. Khalesi M, FitzGerald R.J. Insolubility in milk protein concentrates: potential causes and strategies to minimize its occurrence. *Crit. Rev. Food Sci. Nutr*. 2022a, 62(25), 6973–6989. Khalesi M, FitzGerald R.J. Impact of variation in calcium level on the technofunctional properties of milk protein concentrate. *Colloid Surf A*. 2022b, 643, 128741-128748. Kristensen H.T., Denon Q., Tavernier I., Gregersen S.B., Hammershøj M., Van der Meeren P., Dewettinck K. and Dalsgaard T.K. Improved food functional properties of pea protein isolate in blends and co-precipitates with whey protein isolate, *Food Hydrocolloid*.2021, 113, 106556. Le Roux L., Mejean S., Chacon R., Lopez C., Dupont D., Deglaire A., Nau F., Jeantet R. Plant proteins partially replacing dairy proteins greatly influence infant formula functionalities. *LWT Food Sci. Technol*. 2020, 120, 108891. Malaki Nik A., Tosh S.M., Woodrow L., Poysa V., Corredig M. Effect of soy protein subunit composition and processing conditions on stability and particle size distribution of soymilk. *LWT Food Sci. Technol*. 2009, 42(7), 1245–1252. Molina E., Papadopoulou A., Ledward D.A. Emulsifying properties of high pressure treated soy protein isolate and 7S and 11S globulins. *Food Hydrocolloid*. 2001, 15(3), 263–269. Nadathur S.R., Wanasundara J.P.D., Scanlin L. *Sustainable Protein Sources*, London, Oxford, California, Massachusetts: Elsevier. 2017. Nikbakht Nasrabadi M., Sedaghat Doost A., Mezzenga R. Modification approaches of plant-based proteins to improve their techno-functionality and use in food products. *Food Hydrocolloid*. 2021, 118, 106789–106811. Onwulata C.I., Tunick M.H., Mukhopadhyay S. Flow behavior of mixed-protein incipient gels. *Int. J. Food Prop*. 2014,17(6), 1283–1302. Pizones Ruiz-Henestrosa V. M., Martinez M. J., Carrera Sánchez C., Rodríguez Patino J. M., Pilosof A.M.R. Mixed soy globulins and  $\beta$ -lactoglobulin systems behaviour in aqueous solutions and at the air–water interface. *Food Hydrocolloid*. 2014,35(Supplement C), 106–114. Reidy P.T., Walker D.K., Dickinson J.M., Gundermann D.M., Drummond M.J., Timmerman K.L., Fry C.S., Borack M.S., Cope M.B., Mukherjea R., Jennings K., Volpi E., Rasmussen B.B. Protein blend ingestion following resistance exercise promotes human muscle protein synthesis. *J. Nutr*. 2013, 143, 410–416. Reinkensmeier A., Bußler S., Schlüter O., Rohn S., Rawel H.M. Characterization of individual proteins in pea protein isolates and air



classified samples. *Food Res. Int.* 2015, *76*, 160–167. Singh T., Sogi D. Comparative study of structural and functional characterization of bran protein concentrates from superfine, fine and coarse rice cultivars. *Int. J. Biol. Macromol.* 2018, *111*, 281–288. Tarrega A., Ramírez-Sucre M.O., Vélez-Ruiz J.F., Costell E. Effect of whey and pea protein blends on the rheological and sensory properties of protein-based systems flavoured with cocoa. *J. Food Eng.* 2012, *109*, 467–474. Tian S., Kyle W., Small D. Pilot scale isolation of proteins from field peas (*Pisum sativum* L.) for use as food ingredients. *Int. J. Food Sci. Technol.* 1999, *34*(1), 33–39. United Nations, World Population Prospects. (2019). <https://population.un.org/wpp/> Webb M.F., Naeem H.A., Schmidt, K.A. Food protein functionality in a liquid system: A comparison of deamidated wheat protein with dairy and soy proteins. *J. Food Sci.* 2002, *67*(8), 2896–2902. Wegrzyn T.F., Acevedo-Fani A., Loveday S.M., Singh H. *In vitro* dynamic gastric digestion of soya protein/milk protein blended beverages: influence of protein composition and co-processing. *Food Funct.* 2021, *12*(6), 2605–2616. Xia N., Wang J.M., Gong Q., Yang X-Q., Yin S-W., Qi J.R. Characterization and *in vitro* digestibility of rice protein prepared by enzyme-assisted microfluidization: Comparison to alkaline extraction. *J. Cereal Sci.* 2012, *56*(2), 482–489. Yerramilli M., Longmore N., Ghosh S. Improved stabilization of nanoemulsions by partial replacement of sodium caseinate with pea protein isolate *Food Hydrocolloid.* 2017, *64*, 99–111.

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