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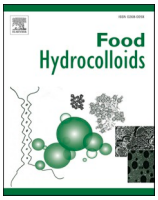

Synergistic adsorption of surface-active components at the air-liquid interface improves foaming properties of plant-based egg analogues

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ABSTRACT

Foaming is an important functionality of eggs in aerated food products. However, plant proteins, the main components of plant-based egg analogues, are generally inferior in foaming compared to egg proteins. Hence, this study aimed to elucidate the foaming mechanisms in plant-based egg analogues modified by different amounts of hydroxypropyl methylcellulose (HPMC). The molecular adsorption at the air-liquid interface (ALI) with the mass transfer coefficients (k) was determined by a two-film theory. Plant-based egg analogue with 1.5% HPMC addition matched the foaming capacity (231% vs. 227%) and foaming stability (72% vs. 73%) of liquid egg, due to the thermodynamic incompatibility between biomacromolecules in the vicinity of the ALI that accelerated the protein and lipid adsorption through depletion mechanism (23.2 and 22.7 to −67.8 μm/min for $k_{protein}$ and k_{lipid}, respectively). This led to the highest volumetric synergy, consistency, and elasticity in the foams made thereof. Foams containing less HPMC did not have fast molecular adsorption, probably due to less HPMC on inducing synergistic adsorption. Foams contained more HPMC exhibited HPMC-dominated ALI, competing with protein and lipid adsorption, and reducing foaming properties. It is concluded that HPMC imparts dose-dependent effect on the molecular adsorption at the ALI, which determines the foaming properties of the plant-based egg analogues. Furthermore, this study showed that plant-based egg analogue modified with 1.5% HPMC was promising in sponge cake and omelet production. Accordingly, a series of composite eggless systems based on foaming characteristics can be developed by adjusting the additive amount of HPMC according to actual needs.

1. Introduction

Egg is an essential ingredient that imparts multi functionalities to meet the requirements of various foods. These functionalities are believed to contribute to the customers' experience with the final product in many aspects, such as nutritional properties (e.g., protein fortification) sensory properties (e.g., mouthfeel, flavor and aroma, browning, etc.), and functional properties (e.g., foam stabilization, binding, thickening, etc.). Among them, the excellent foaming properties exerted by egg white proteins provide the light mouthfeel for various foam products, including omelets, sponge cake, soufflés, meringues, etc. These properties are dependent on proteins' abilities to adsorb rapidly at the air-liquid interface (ALI) during whipping, and on their abilities to develop the viscoelastic films through intermolecular interactions (Mine, 1995). During whipping, air is trapped into the egg liquid as bubbles. The hydrophobic regions of proteins promote their adsorption at the ALI, followed by partial unfolding (surface denaturation) of the adsorbed proteins (Liu, Oey, Bremer, Carne, & Silcock, 2019). Then these partially unfolded proteins associate to surround the bubbles, which is crucial to foam stability (Kinsella, 1981; Liu et al., 2019). Interactions between proteins enhance the cohesion of the films, thereby imparting elasticity and stability to the foams (Kinsella, 1981; Mine, 1995). Albumen contains a mixture of proteins that exert different functions (Stadelman, Newkirk, & Newby, 2017)- globulin is a good former, but its foaminess is dependent on its interactions with ovomucin, lysozyme, ovomucoid, ovotransferrin, and ovalbumin.

In the plant-based egg analogues, plant proteins stabilized the ALI by adsorbing, unfolding, and developing a viscoelastic film around the bubbles (Huang, Zhang, Ding, Wu, & Li, 2020; Patino et al., 2007). However, these functionalities should differ from those of the egg

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proteins, owing to their different protein composition (Amagliani, Silva, Saffon, & Dombrowski, 2021), different solubility in the liquid phase, different abilities to adsorb at the ALI (Fox & Condon, 1982), and different abilities to involve in molecular interactions (Söderberg, 2013). Unlike the viscoelastic films formed by proteins, lipids develop a more fluid-like film at the ALI and stabilize the ALI based on the Gibbs-Marangoni mechanism. However, these surface-active lipids could be gradually redistributed at the ALI to attain equilibrium conditions (Damodaran, 2005; Murray, 2007). Indeed, the roles of proteins and lipids at the ALI are inherently opposite, where proteins show a viscoelastic solid-like behavior while lipids show a more fluid-like behavior (Mackie & Wilde, 2005; Murray, 2007; Pycarelle, Bosmans, Nys, Brijs, & Delcour, 2020). Such antagonism may limit the air cell incorporation and stability in the plant-based egg analogues.

Hydroxypropyl methylcellulose (HPMC) is a good film former, suspension agent, emulsifier, thickener, and non-toxic food additive that is widely used in food industry (Zhang, Yu, Wang, & Wu, 2018). Previous studies have evaluated the interfacial properties of the milk protein-HPMC systems, and have pointed out that when milk proteins adsorb at the ALI in the presence of HPMC, three phenomena could occur: 1) HPMC adsorbs at the ALI in competence with milk proteins (competitive adsorption); 2) HPMC interacts with milk proteins through electrostatic interactions or hydrogen bonding; 3) HPMC concentrates milk protein adsorption due to the limited thermodynamic compatibility (i.e., limited protein-polysaccharide complexing and promoted association between macromolecules of the same type) in the vicinity of the ALI (Baeza, Sanchez, Pilosof, & Patino, 2005; Perez, Martinez, Carrera; Pérez, Sánchez, Pilosof, & Patino, 2009; Sanchez, & Patino, 2017). The occurrence of these phenomena depends on the conditions of the systems as well as the bulk concentration of each biopolymer within the systems. In the current study, the pH of the plant-based egg analogues was above the protein isoelectric point, thus phenomenon 3) could be more preferred due to the repulsive electrostatic interactions between soy proteins (zeta-potential = −21.79 mV) and HPMC (zeta-potential = −1.76 mV), and their various affinities for the solvent (Damodaran, 2017). In this case, proteins and polysaccharides may coexist in the same phase (i.e., miscibility) in the diluted-concentration-region, but mutually exclude one another in domains and eventually segregate into different phases above the critical concentration (Martinez, Sanchez, Ruiz-Henestrosa, Patino, & Pilosof, 2007). It was well studied that limited thermodynamic compatibility between proteins and polysaccharides promoted their interactions at the fluid interfaces (Baeza et al., 2005; Carp, Bartholomai, & Pilosof, 1999; Martinez et al., 2007; Pérez et al., 2009; Pérez, Martinez, Sánchez, & Patino, 2017). For instance, it was shown that limited thermodynamic compatibility between soy proteins and xanthan gum existed under neutral pH, which facilitated the aggregation of soy protein subunits at the ALI (Carp et al., 1999). This could further affect the interfacial and foaming properties of the protein-polysaccharide system. To the best of our knowledge, there is limited information reported on the foaming properties of the plant-based egg analogues, in which surface-active components (e.g., HPMC, proteins, and lipids) could determine the interfacial properties.

Therefore, this study aimed to modify the foaming properties of the plant-based egg analogues with HPMC, and more importantly, to explore the foaming mechanisms within the plant-based egg analogue systems. For these purposes, the molecular population adsorbed at the ALI in the plant-based egg analogues was first investigated based on a foam separation protocol (Pycarelle et al., 2020). Then the molecular transfers over time were described by a two-film theory, and the obtained mass transfer coefficients were related to the rheological properties of the foams as well as the foaming properties of the plant-based egg analogues. Furthermore, the plant-based egg analogues were applied in two aerated food products (i.e., omelets, sponge cakes) to evaluate their effectiveness on egg replacement. This work can provide new insights in the functionality of surface-active ingredients in the plant-based egg analogue systems, which is conducive to the development of stabilized food foams.

2. Materials and methods

2.1. Materials

Myprotein® soy protein isolate, Windmill® potato starch, Naturel® sunflower oil, Dr Gram® chickpea flour, RedMan® baking powder, Usolf® hydroxypropyl methylcellulose (4000 mPa s, HPMC), Prima flour® cake flour, RedMan® sponge cake gel (main ingredients: sorbitol, propylene glycol, and emulsifier (E471, E475)), and Flying Man® fine grain pure cane sugar, were purchased from the online shops of Lazada, Singapore. N&N® liquid egg, procured from N&N Agriculture Pte Ltd, was set as the benchmark. Other food-grade ingredients included Ajinomoto® transglutaminase (Amazon, Seattle, USA), potassium chloride (Sigma-Aldrich, Singapore), and κ-carrageenan (Better 4U Holdings Pte Ltd, Singapore). All these ingredients were of food-grade.

Copper sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$), potassium sulfate (K_2SO_4), sulfuric acid (H_2SO_4), boric acid (H_3BO_3), hydrochloric acid (HCl), petroleum ether, potassium bromide (KBr), calcofluor white, Nile red, and fluorescein isothiocyanate (FITC) were obtained from Sigma-Aldrich (Singapore). Sodium hydroxide (NaOH) was obtained from VWR Singapore Pte Ltd. (Singapore). All these chemicals were of analytical-grade.

2.2. Preparation of plant-based liquid egg analogues

In our previous study, we have optimized the gelling functionality as well as the physicochemical properties of the plant-based egg analogues using different starches (Lu et al., 2023). However, an inferior foaming property of the plant-based egg analogues in comparison to the authentic liquid egg was reported regardless of the starch addition (Lu et al., 2023). To improve the foaming properties of the current plant-based liquid egg analogues, the formulations were modified with the addition of 4000 mPa s hydroxypropyl methylcellulose (HPMC) at various concentrations (0.0%, 1.0%, 1.5%, 2.0%, (w/w)). Then all the ingredients, including soy protein isolate (8.82%), chickpea flour (3.78%), potato starch (6.0%), sunflower oil (5.6%), baking powder (1.0%), transglutaminase (0.025%), κ-carrageenan (0.3%), potassium chloride (0.1%), and 0.0%, 1.0%, 1.5%, or 2.0% HPMC solution (74.375%), were blended with an electric blender (Aztech, Singapore) at speed 1 (20,000 rpm) for 3 min to prepare the plant-based liquid egg analogues.

2.3. Foaming properties of liquid egg and plant-based egg analogues

The foaming properties of the control liquid egg and the plant-based liquid egg analogues were evaluated according to Pycarelle et al. (2020) with modifications. To be specific, 30.00 g of liquid egg and 96.25 g of DI water were mixed in a beaker covered by parafilm with a magnetic stirrer at 25 °C for 30 min. The suspension was then transferred into a measuring cylinder (internal diameter = 60.0 mm). The initial liquid volume was recorded (V_0), after which the suspension was whipped with an electric whisk (MSM66150, Bosch, Singapore) that was lowered to the bottom of the measuring cylinder at 2000 rpm for 2.5 min. The whisk was carefully elevated, and the measuring cylinder was sealed with parafilm to reduce air circulation. The volume of the foam was recorded over 60 min, during which foaming capacity was calculated as:

$$FC(\%) = \frac{V_{3.5}}{V_0} \times 100\% \quad (1)$$

where $V_{3.5}$ is the foam volume recorded at 3.5 min after the start of whipping and V_0 is the initial liquid volume. Foaming stability was calculated as:

$$FS(\%) = \frac{V_{60}}{V_{3.5}} \times 100\% \quad (2)$$

where V_{60} is the foam volume remaining at 60 min after the start of whipping.

2.4. Synergistic effects of the components on the foam volumes over time

The synergic interactions taking place in the plant-based egg analogue mixtures involving HPMC and other constituent components were evaluated according to Herraez and Belda (2004) and Zhu et al. (2021) with modifications. The method characterized the synergic behaviors through comparing the foam volume that was determined experimentally (V_{exp}) with the expected foam volume that was determined by the simple mixing rule (V_{mix}):

$$V_{mix} = w_{HPMC}V_{HPMC} + w_{0HPMC}V_{0HPMC} \quad (3)$$

where w_{HPMC} and w_{0HPMC} are the mass fraction of the HPMC and other plant-based egg analogue components (0HPMC), and V_{HPMC} and V_{0HPMC} are the foam volumes, determined experimentally, of the HPMC and other plant-based egg analogue components (0HPMC), respectively. Furthermore, the synergic interaction index (I_v) was calculated to compare the volumetric synergies within the different plant-based egg analogue systems:

$$I_V = \frac{V_{exp} - V_{mix}}{V_{mix}} \quad (4)$$

To further elucidate the synergic interactions between HPMC and soy protein isolate (SPI) or sunflower oil (SO) within the plant-based egg analogue systems, the synergic interaction index was also determined for the SPI-free (SPI(-)SO(+)), SO-free (SPI(+)SO(-)), and SPI-, SO-free systems (SPI(-)SO(-)).

2.5. Foam separation and foam yield

After whipping for 2.5 min, the foam was isolated at T1 (3.5 min after whipping starts), T2 (15 min after whipping starts), and T3 (60 min after whipping starts), respectively. Both the diluted liquid eggs and the isolated foams were lyophilized for 7 days in a freeze-dryer (Buchi, Singapore). All the lyophilized samples were weighted and ground, after which the foam yield at each time point was calculated as:

$$Foam\ yield\ (\%) = \frac{foam\ dry\ matter\ (g)}{liquid\ egg\ dry\ matter\ (g)} \times 100 \quad (5)$$

2.6. Image analysis of liquid egg, plant-based liquid egg analogues, and foams made thereof

The distributions of gas cell size in the foams taken at different time point (T1, T2, T3) were qualitatively evaluated with an Olympus SZ61 (Olympus, Melville, NY, USA) stereomicroscope attached with a Nikon DS5100 digital camera (Nikon Corporation, Tokyo, Japan). Images were captured at 10× magnification in the NIS-Elements BR software (Nikon). The microscopy images were processed in the ImageJ2 software (National Institutes of Health, USA) to determine the average size of air bubbles.

2.7. Rheological properties of liquid egg, plant-based liquid egg analogues, and foams made thereof

Rheological measurements were performed with a rotational stress-controlled rheometer (MCR 102, Anton Paar, Graz, Austria) fitted with a stainless-steel cone plate (diameter = 25 mm, gap = 1.0 mm). The diluted liquid egg, plant-based liquid egg analogues, and foams made thereof were transferred onto the rheometer platform at 25 °C for the following testing:

(1) a steady shear flow test over the shear rate of 0.001–1000 s^{-1};

(2) an amplitude oscillatory sweep from 0.1 to 1000% at the frequency of 1 Hz and the temperature of 25 °C.

2.8. Compositions of liquid egg, plant-based liquid egg analogues, and foams made thereof

The protein contents of the lyophilized liquid egg, plant-based liquid egg analogues, and foams were determined with the Kjeldahl method in a FOSS Kjeltec System (AN300, FOSS, Denmark). The general nitrogen-to-protein conversion factor (6.25) reported by Mariotti, Tomé, and Mirand (2008) was used for all samples. The protein recovery, reflecting the portion of proteins that was initially present in the plant-based liquid egg analogues ended up in the foams, was calculated as:

$$Protein\ recovery\ (\%) = \frac{foam\ yield\ (\%) \times foam\ protein\ content\ (\%, d.b.)}{liquid\ egg\ protein\ content\ (\%, d.b.)} \quad (6)$$

The lipid contents of the lyophilized liquid egg, plant-based liquid egg analogues, and foams were gravimetrically determined after Soxhlet extraction with petroleum ether under room temperature in a Gerhardt Fat Extraction System (Gerhardt, Singapore). The lipid recovery was calculated to show the amount of lipids recovered in the foams from the plant-based liquid egg analogues.

$$Lipid\ recovery\ (\%) = \frac{foam\ yield\ (\%) \times foam\ lipid\ content\ (\%, d.b.)}{liquid\ egg\ lipid\ content\ (\%, d.b.)} \quad (7)$$

The protein-to-lipid ratio was calculated as:

$$Protein - to - lipid\ ratio = \frac{foam\ protein\ content(\%, d.b.)}{foam\ lipid\ content(\%, d.b.)} \quad (8)$$

2.9. Determination of protein and lipid mass transfer

The concentrations of protein and lipid in the foams isolated at different time points were determined as:

$$C_{foam} = \frac{foam\ dry\ weight \times foam\ protein\ or\ lipid\ content\ (\%, d.b.)}{volume\ of\ liquid\ in\ foam} \quad (9)$$

Then the concentrations were fitted into the logarithmic equation (Kaplanow, Goerzgen, Merz, & Schembecker, 2019) and exponential decay equation (Zhang et al., 2020) with modifications:

$$Q = \ln\frac{c_{foam,\infty} - c_{foam,0}}{c_{foam,\infty} - c_{foam}} \bullet \frac{V_{foam}}{A_{cell}} = \begin{cases} klnt \\ a + be^{kt} \end{cases} \quad (10)$$

where $c_{foam,\infty}$ is the final concentration in the foam phase (here the concentration at 2 h), $c_{foam,0}$ is the concentration in the foam phase at the beginning (t = 0), c_{foam} is the concentration in the foam phase at time t, V_{foam} is the volume of the foam phase, and A_{cell} is the interfacial area. Through the logarithmic or exponential fitting of Q plotted against time, the mass transfer coefficients (k) were extracted to describe the mass transfer through the ALI.

2.10. FTIR analysis of liquid egg, plant-based liquid egg analogues, and foams made thereof

The lyophilized liquid egg, plant-based liquid egg analogues, and foams were subjected to FTIR analysis as described by Lu, Lee, and Yang (2022). Briefly, 3 mg of sample powders was ground with 97 mg of KBr to prepare the pellets. Then the FTIR spectra (4000-450 cm^{-1}) were collected at the resolution of 4 cm^{-1}, and the scan number of 32, in a Spectrum One FTIR spectrometer (PerkinElmer, Waltham, MA, USA) after correcting for the background spectrum. The amide I region (1700-1600 cm^{-1}) was Fourier self-deconvoluted in the Omnic 8.2 software (Thermo Fisher Scientific Inc. Waltham, MA, USA), and the Gaussian peak fitting was performed in the OriginPro 9.0 software (OriginLab, Northampton, MA, USA). The distribution of protein

secondary structures was reflected by the relative integrated area of the fitted peak.

2.11. CLSM analysis of liquid egg, plant-based liquid egg analogues, and foams made thereof

The diluted liquid egg and plant-based liquid egg analogue samples were fluorescently labelled according to Huang, Mao, Li, and Yang (2021) with modifications. FITC, calcofluor white, and Nile red were used to label proteins, polysaccharides, and lipids, respectively. To be specific, the mixture (1:1:1, (v/v/v)) of FITC (0.1 g/L in ethanol), calcofluor white (0.1 g/L in ethanol), and Nile red (0.1 g/L in ethanol) was added into the samples at a ratio of 1:100 (v/v), followed by a 30-min--staining under room temperature. Then the stained samples were transferred onto a cover glass slide for further observation. The fluorescently labelled foams were prepared by whipping the stained liquid egg and plant-based liquid egg analogues with the electric whisk at 2000 rpm for 2.5 min. The foam taken at 3.5 min (T1), 15 min (T2), and 60 min (T3) was smeared onto the cover glass slide immediately. An Olympus Fluoview FV1000 confocal scanning unit (Tokyo, Japan) with argon ion and helium–neon laser was used to observe the microstructures of the liquid egg, plant-based liquid egg analogues, and foams made thereof. The excitation/emission wavelengths of FITC, calcofluor white, and Nile red were 490/525 nm, 380/475 nm, and 559/635 nm, respectively. Images were collected at 10× magnification and processed in Imaris software (9.2.0, Bitplane, Oxford Instruments, Oxfordshire, UK).

2.12. Application of plant-based liquid egg analogues in omelet dish and sponge cake

The omelet dish and sponge cake prepared with the control liquid egg, or the plan-based liquid egg analogues were made as published earlier (Huang & Yang, 2019; Lu et al., 2022). For the American-style omelet preparation, 100 g of liquid egg was heated in a pre-heated frypan (Lamart, Singapore) without stirring (130 °C, 2.5 min), then flipped to continue heating (1 min), and cooled for further analyses (room temperature, 60 min). For the sponge cake preparation, 200 g of liquid egg, 100 g of sugar, and 8 g of cake emulsifier were firstly blended thoroughly (180 rpm, 8 min) in a mixer (KitchenAid, St Joseph, MI, USA). Then 100 g of cake flour and 25 g of sunflower oil were added and homogenized (60 rpm, 2 min). Afterwards, 250 g of the cake batter was poured into a cylindrical baking tin, and baked in a convection oven (Fabricant Eurfours, Gommegnies, France) (180 °C, 30 min). The cakes were cooled for further analysis (room temperature, 60 min).

The properties of omelet pieces (cut by a ring mold with diameter = 20 mm) and sponge cakes were evaluated as in Huang and Yang (2019). The volume was determined by scanning five stacking omelet pieces or the whole cake in a Stable Microsystem Volscan profiler 600 (Stable Micro Systems Ltd., Surrey, UK). The specific volume was calculated as:

$$\text{Specific volume} = \frac{Volume}{Mass} \tag{11}$$

The textural profile was determined for the omelet pieces and the cake cubes (cut into 20 mm × 20 mm × 20 mm) using a TA-XT2i texture analyzer (Stable Micro System Ltd., Surrey, UK) equipped with a P/35 probe. A double compression test was performed with the pre-test speed of 5 mm/s, the test speed of 1 mm/s, the post-test speed of 1 mm/s, and the compression deformation of 50% of the initial sample height. Several textural attributes, including hardness, cohesiveness, springiness, chewiness, and resilience, were determined through the force-time curves.

2.13. Statistical analysis

All analyses were conducted independently at least in triplicate. At least ten parallel stereomicroscope and CLSM images were collected for each sample. All results were expressed in mean or mean ± S.D. Data were tested in the SPSS Statistics 20 software (IBM, Chicago, IL, USA) using one-way ANOVA and post-hoc Bonferroni test, and $P < 0.05$ (two-tailed) was regarded as statistically significant among groups.

3. Results and discussion

3.1. Foaming properties

The changes in foam volume over time are depicted in Fig. 1A. For both the liquid egg and the plant-based liquid egg analogues, the foam volume decreased as time increased, which could be attributed to the coarsening of foam, and the increasing drainage of bulk fluid from foam to liquid phase (Pycarelle et al., 2020; Saint-Jalmes, 2006). The plant-based liquid egg analogue with no HPMC modification (0HPMC) had significantly lower initial foam volume (T1) than that of the control liquid egg (LE), indicating the inferior foaming capacity of 0HPMC (Fig. 1B). The initial foam volume and foaming capacity of the plant-based liquid egg analogues were improved significantly with the increasing HPMC addition, of which the concentration at 1.5% had attained the foaming properties of the control liquid egg (Fig. 1B). This could be attributed to the gradually elongating loops and tails due to the increasing adsorption of hydrophobic segments at ALI (Narsimhan, 2016; Zhu et al., 2021). When the HPMC addition reached 2%, the interfacial films may be entirely covered by the absorbed hydrophobic groups. Moreover, the overall viscosity of the 2HPMC did not differ significantly from that of the 1.5HPMC (Fig. 2C), thus the foaming capacity remained unchanged (Fig. 1B). This observation was in line with the foaming behavior of the HPMC-welan gum mixture at the ratio of 2:8, which showed a constant foam volume after reaching the concentration of 1%.

In addition, despite the comparable foam volume of 1.5HPMC and 2HPMC with LE within 15 min, only 1.5HPMC could maintain a foam volume equivalent to LE at T3 (Fig. 1A). This corresponded to the similar foaming stability of plant-based liquid egg analogues with HPMC addition at 1.5% to LE, as shown in Fig. 1B. It indicated that the addition of HPMC greatly enhanced the foaming stability of the plant-based egg analogue systems, possibly involving the synergistic effects between HPMC and other plant-based egg analogue components on limiting foam destabilization and drainage (Tan, 2019). To further explore the foam stability at the micro-level, the morphological images of LE and plant-based egg analogue foams at different time points are presented (Fig. 1C). The air bubbles tended to be more compact and uniform (i.e., initial average diameter decreased from 221 to 178 μm; Fig. 1D) with the increasing addition of HPMC from 0% to 1.5%, and the initial average bubble diameter of the 1.5HPMC foam was comparable to that of the LE foam (178 vs 168 μm). Similar observations were also reported in the whey protein foam (Borcherding, Lorenzen, & Hoffmann, 2009) and the xanthan gum-whey protein foam (Martínez-Padilla, García-Rivera, Romero-Arreola, & Casas-Alencáster, 2015), where the diameter of air bubbles decreased with the increasing concentration of whey protein and xanthan gum-whey protein, respectively. The change in bubble size along with HPMC additive amount followed a parallel trend to the foam stability (Fig. 1B), probably because the more uniform bubbles have a higher surface tension and a lower buoyancy force, which allows the gas of the smaller bubbles dissolve quicker and coalescence slower (Zhu et al., 2021). The diameters of the LE and HPMC-added foams increased over time, while that of the non-HPMC-added foam decreased over time (Fig. 1D). This was associated with the poor stability of the 0HPMC foam: most of the bubbles burst within 60 min, leaving only a few small bubbles observed at T3 (Fig. 1C). In contrast, the plant-based egg analogues modified with HPMC can hold a relatively more stable foam even after the storage of 60 min (T3), albeit the smaller bubble sizes compared to the LE foam (Fig. 1D). In this case, some of the bubbles still coalesced and rose due to the buoyancy force and gravity, leading to a

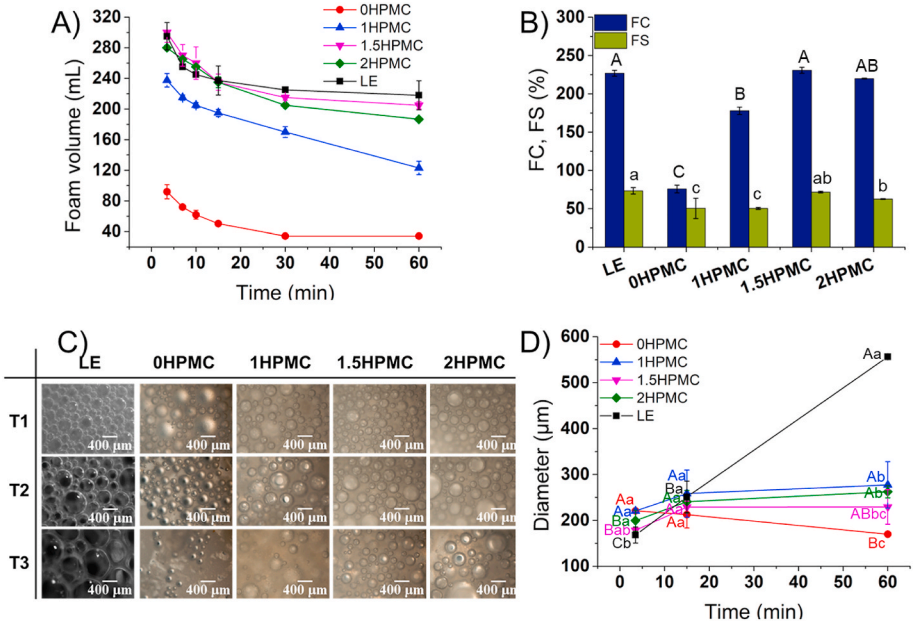

Fig. 1. Foaming properties of plant-based egg analogues in comparison of liquid egg (LE). A) Changes in foam volume over time; B) Foaming capacities and foaming stabilities; C) Morphological characteristics of the foams isolated at different time point; D) Calculated diameter of the foams changed with time. *At each time point, groups with different lowercase letters indicate significant difference ($P < 0.05$) among samples; For the same sample, groups with different capital letters indicate significant difference ($P < 0.05$) among different time point. *0HPMC, 1HPMC, 1.5HPMC, and 2HPMC refer to plant-based liquid egg analogues with addition of 0%, 1%, 1.5%, and 2% HPMC (w/w), respectively. *FC: foaming capacity, FS: foaming stability.

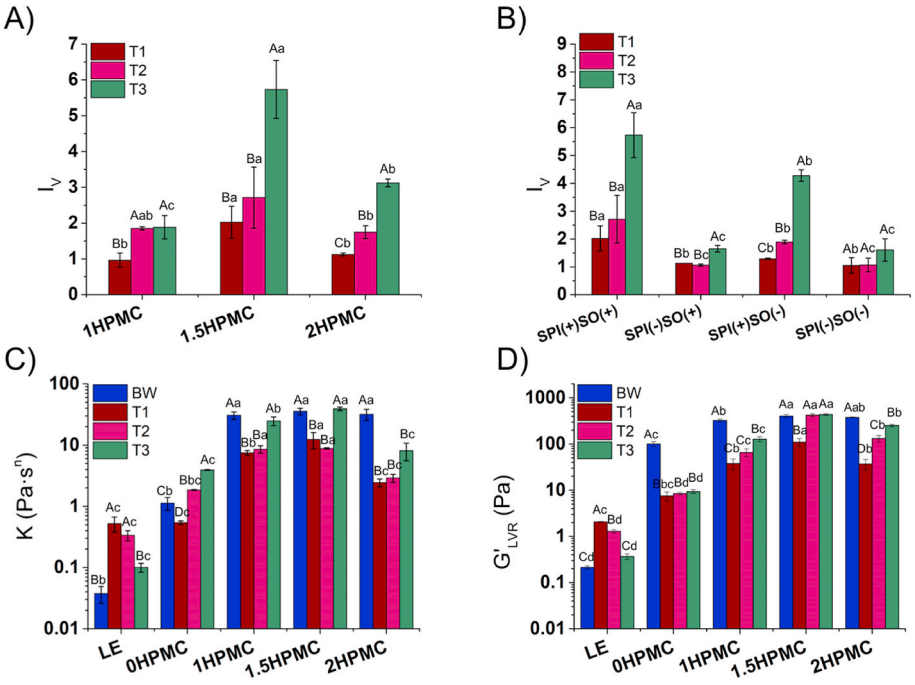

Fig. 2. A) Volumetric synergy between HPMC and the plant-based egg analogue system; B) Volumetric synergy between 1.5% HPMC and the plant-based egg analogue system in the presence/absence of soy protein isolate (SPI) or sunflower oil (SO); C) Consistency coefficients of liquid egg (LE), plant-based egg analogues, and foams made thereof; D) Storage modulus within the linear viscoelastic region (G'_{LVR}) over the strain sweep. *At each time point, groups with different lowercase letters indicate significant difference ($P < 0.05$) among samples; For the same sample, groups with different capital letters indicate significant difference ($P < 0.05$) among different time point. *0HPMC, 1HPMC, 1.5HPMC, and 2HPMC refer to plant-based liquid egg analogues with addition of 0%, 1%, 1.5%, and 2% HPMC (w/w), respectively. *BW, T1, T2, and T3 refer to the diluted plant-based liquid egg analogues before whipping, foams isolated at T1 (3.5 min), T2 (15 min), and T3 (60 min), respectively.

decrease in foam volume over time for all samples (Fig. 1A). The main difference was observed for the bubble morphology at T3. In the storage process, air bubbles coalesced and formed larger bubbles, during which some larger bubbles in the HPMC-modified plant-based egg foams burst, while those in the LE foams could be held. This indicated that the LE foams were stable, which was consistent with the results in Fig. 1B. For the 1.5HPMC foams, although no larger bubbles were observed at T3, they were uniformly and closely distributed. This may contribute to their similar foam volume and foam stability as LE.

Furthermore, foam yields were determined to evaluate the dry matter transfer during the incorporation and stabilization of gas cells (Table 1). For all the liquid egg samples, foam yields decreased significantly with time, mainly due to the drainage of dry matter from foams to liquids (Kruglyakov, Elaneva, & Vilkova, 2011; Pycarelle et al., 2020). For the foams isolated from the plant-based egg analogues, foam yields were significantly enhanced with the increasing addition of HPMC at T1 and T2, while at T3, yield of foam prepared from 1.5HPMC was the highest. This evidenced the lowest loss of dry matter from the 1.5HPMC foam at T3, which may contribute to its foam stability (Fig. 1B).

3.2. *Synergistic effects of the components on the foaming properties of the plant-based liquid egg analogues*

The synergistic effects between HPMC and the plant-based egg analogues on the foam volumes over time are displayed in Fig. 2A. As can be seen, the addition of 1.5% HPMC induced the highest synergistic effect with the plant-based egg analogue system on foaming, and this effect increased along with the storage time. Upon increasing HPMC

Table 1
Foam yields, protein contents, lipid contents, and protein-to-lipid ratios of liquid egg, plant-based egg analogues, and foams made thereof.

		BW	T1	T2	T3
Foam yield (%)	LE	–	53.1 ± 2.3^{Ab}	21.0 ± 0.9^{Bd}	8.9 ± 0.4^{Cb}
	0HPMC	–	37.0 ± 4.4^{Ac}	24.5 ± 1.2^{Bd}	15.0 ± 7.8^{Bb}
	1HPMC	–	91.8 ± 7.0^{Aa}	72.6 ± 3.2^{Bc}	60.2 ± 4.6^{Ba}
	1.5HPMC	–	100.0 ± 0.0^{Aa}	87.1 ± 4.9^{Bb}	69.4 ± 1.4^{Ca}
	2HPMC	–	100.0 ± 0.0^{Aa}	96.2 ± 0.0^{Aa}	64.1 ± 2.3^{Ba}
Protein content (%, d.b.)	LE	51.4 ± 4.7^{Aa}	46.0 ± 2.7^{ABa}	44.8 ± 0.4^{Ba}	44.3 ± 0.6^{Ba}
	0HPMC	33.9 ± 0.2^{Ab}	15.5 ± 1.3^{Cc}	20.5 ± 3.5^{BCc}	22.1 ± 1.2^{Bd}
	1HPMC	32.4 ± 0.2^{Ab}	19.8 ± 1.5^{Cc}	21.4 ± 1.6^{Cc}	29.0 ± 0.5^{Bc}
	1.5HPMC	33.5 ± 1.5^{ABb}	30.6 ± 2.0^{Bb}	32.7 ± 2.3^{ABb}	36.7 ± 1.5^{Ab}
	2HPMC	33.0 ± 1.2^{Ab}	33.2 ± 1.0^{Ab}	24.4 ± 2.3^{Bc}	26.8 ± 1.9^{Bc}
Lipid content (%, d.b.)	LE	41.4 ± 3.5^{Aa}	42.9 ± 5.9^{Aa}	41.9 ± 4.2^{Aa}	35.2 ± 6.6^{Ba}
	0HPMC	13.3 ± 2.4^{Abc}	5.1 ± 0.2^{Bc}	5.1 ± 1.2^{Bc}	14.2 ± 0.5^{Ac}
	1HPMC	12.7 ± 2.0^{Bc}	7.8 ± 1.8^{Cbc}	5.7 ± 0.5^{Cc}	19.1 ± 0.3^{Ab}
	1.5HPMC	20.5 ± 3.8^{Ab}	12.3 ± 3.0^{ABb}	10.2 ± 2.4^{Bb}	17.8 ± 1.6^{ABb}
	2HPMC	17.3 ± 2.7^{Abc}	10.1 ± 1.5^{Bbc}	7.2 ± 2.7^{Bbc}	20.7 ± 0.4^{Ab}
Protein-to-lipid ratio	LE	1.5 ± 0.1^{Ac}	1.1 ± 0.0^{Bb}	1.0 ± 0.0^{Bc}	1.1 ± 0.0^{Bb}
	0HPMC	2.4 ± 0.1^{ABa}	1.2 ± 0.1^{Bb}	4.0 ± 0.7^{Aa}	4.5 ± 1.4^{Aa}
	1HPMC	1.7 ± 0.0^{Cbc}	1.6 ± 0.4^{Cab}	2.8 ± 0.3^{Bab}	5.1 ± 0.2^{Aa}
	1.5HPMC	1.9 ± 0.2^{Bb}	1.5 ± 0.2^{Bab}	2.7 ± 0.6^{ABab}	3.7 ± 0.9^{Aa}
	2HPMC	1.6 ± 0.0^{Cc}	1.9 ± 0.3^{BCa}	2.4 ± 0.2^{Bb}	3.7 ± 0.3^{Aa}

*At each time point, groups with different lowercase letters indicate significant difference ($P < 0.05$) among samples; For the same sample, groups with different capital letters indicate significant difference ($P < 0.05$) among different time point. *LE, 0HPMC, 1HPMC, 1.5HPMC, and 2HPMC refer to liquid egg, and plant-based liquid egg analogues with addition of 0%, 1%, 1.5%, and 2% HPMC (w/w), respectively. *BW, T1, T2, and T3 refer to the diluted plant-based liquid egg analogues before whipping, foams isolated at T1 (3.5 min), T2 (15 min), and T3 (60 min), respectively.

concentration to 2%, the I_V values reduced significantly at each time point; that indicates the decreasing synergistic effect in the 2HPMC foams. This phenomenon could be explained by the adsorption behaviors of HPMC, proteins, and lipids that changed with the HPMC addition. A study on the interfacial properties of HPMC-whey protein concentrate foams has pointed out the dose-dependent kinetics of molecular adsorption (Pérez et al., 2009)- when proteins saturated the ALI and HPMC was at the lowest bulk concentration, proteins were adsorbed in preference to HPMC at ALI. A synergistic behavior in the diffusion rates was seen in the systems where neither HPMC nor WPC could saturate the ALI. With more HPMC added, its adsorption was accelerated, and thus it could dominate the interfacial behaviors before the significant protein adsorption. Also, this surface rheology of the HPMC-dominated interface was great enough that the significant replacement of HPMC by proteins was hardly possible (Mackie, Gunning, Wilde, & Morris, 1999). In addition, HPMC at ≤ 1.5% can induce partial coalescence of fat droplets (Fig. 6) by displacing the proteins at the oil-liquid interface (Zhao et al., 2009). This process plays a vital role in the formation of structure in the whipped foams-a cross-linking of fat globules surrounding each gas cell to adjacent gas cells can effectively stabilize the foam through the developed infra-structure, which has been widely observed in heavy cream (Brooker, Anderson, & Andrews, 1986; Kalab, 1985; Schmidt & Van Hooydonk, 1980). In the current study, the lower synergy in 2HPMC foams indicated the dominant role of HPMC at ALI that repelled the adsorption of proteins and partially coalesced lipids. This could reduce the viscosity and viscoelasticity of the foams (Fig. 2C and D), resulting in a lower foaming stability in the 2HPMC case (Fig. 1B).

To further explore the synergistic effects between 1.5% HPMC and the plant-based egg analogue components, the I_V values were determined for the plant-based egg analogue samples prepared with soy protein isolate (SPI(+)) or without (SPI(-)), and with sunflower oil (SO (+)) or without (SO(-)) (Fig. 2B). In the foam systems with SPI but without SO (SPI(+)SO(-)), volumetric synergism decreased significantly compared to SPI(+)SO(+) (1.5HPMC) foams at any moment. These decreases in synergy values reflected the significant synergistic effects between HPMC-SO in the 1.5HPMC plant-based egg analogue foams. For the systems with SO but without SPI (SPI(-)SO(+)) and the systems free of SPI and SO (SPI(-)SO(-)), the I_V values were further reduced. This behavior further evidenced the role of HPMC-SPI synergy in the volume of the 1.5HPMC foams, which was more significant than the HPMC-SO synergy.

3.3. *Rheological properties of liquid egg, plant-based liquid egg analogues, and foams made thereof*

To show the flow performance of liquid egg, plant-based liquid egg analogues and foams made thereof, the steady shear data were fitted in the power law model, with the consistency coefficient K (Pa·s^n) summarized in Fig. 2C. These consistency coefficients reflect the average viscosity of the systems (Pang, Wang, Chen, Hassan, & Lu, 2020). For the LE, whipping increased the K value significantly, while the K values of the foams decreased gradually from T1 to T3. This could be attributed to the intermolecular disulfide bonds formed by the unfolded ovalbumin molecules at the ALI (Kinsella, 1981; Liu et al., 2019) that may hinder the flow of bubbles. Interestingly, for all the plant-based egg analogue samples, the flow behaviors were contrast-the K values decreased sharply at 1 min after whipping (T1), indicating that the plant-based egg analogues in the foam state were more likely to flow and deform than those in the solution state (BW) due to the high-speed shearing (whipping) process. It might be owing to the more fluids in the gap between the air bubbles observed in the plant-based egg foams than those in the LE foams (Fig. 1C). At T2 (12.5 min after whipping), the 0HPMC foam can recover to a comparable viscosity level to the 0HPMC solution (0HPMC-BW), while the foams made from the HPMC-added samples were still at low viscosity as those measured at T1. The viscosity of the foams continued to increase at T3 when a sharp increase in K values was observed for all the plant-based egg analogue foams. As for the influence of HPMC, the K values were the highest for the 1.5HPMC foams at T1, T2, and T3; this contributed to its superior foam stability (Fig. 1B). It could be suspected that with the increase in HPMC addition, the molecular entanglement between HPMC and the components in the plant-based egg systems increased, which could enhance the cross-linking between bubbles (indicated by the more uniform bubble distribution at higher HPMC (Fig. 1C); Xu, Xu, Liu, Chen, & Gong, 2013; Zhu et al., 2021), limit the flow of bubbles, and thus increase the viscosity of the foams. However, 2% HPMC appeared to dominate the ALI and compete with other surface-active components for the adsorption, leading to the lower viscosity of the 2HPMC foams. Such lower viscosity was responsible for the lower foam stability of the 2HPMC (Fig. 1B) as it was less able to restrain the gas diffusion and/or retard the film drainage (Li, Pu, & Chen, 2022).

It was also noted that the cross-linking and entanglement of the aggregates in the bulk phase and the surface structures of the bubbles could contribute to the viscoelastic properties of the foams (Peng, Jin, Sagis, & Li, 2022). To further elucidate the viscoelastic behaviors of LE

and different plant-based egg analogue foams isolated at different time points, the G′ values within the LVR (G'_{LVR}) are summarized in Fig. 2D. Similar to the flow performance mentioned above, whipping increased the elastic response of the LE foams, and such increase decreased over the storage time. However, for the plant-based egg analogues, high-speed whipping may disrupt the gel structures formed before whipping, leading to a significant decrease in G'_{LVR} of foams at T1. With the longer storage time, the elasticity of foams gradually recovered. For the HPMC impact, increasing the HPMC addition from 0% to 1.5% induced a remarkable increase in G'_{LVR} values for foams isolated at every time point, suggesting a significant increase in foam elasticity. The G'_{LVR} of foams clearly decreased with a further increase in HPMC addition to 2%. Foams generated from 1.5HPMC possessed the highest modulus within the LVR, illustrating that these foams formed more elastic and rigid structures than those of other formulations. This may be the result of the increased cross-linked structures on the bubble surfaces observed at that concentration (Fig. 6).

3.4. Quantification of proteins and lipids in liquid egg, plant-based liquid egg analogues, and foams made thereof

Due to the large interfacial areas of the foams prepared from the diluted liquid egg and plant-based liquid egg analogues, it was suspected that the affinity of the molecules residing in the foams for ALI was higher than that for bulk liquid phase (Pycarelle et al., 2020; Schramm & Wassmuth, 1994). Based on this assumption, the molecules enriched in the foams may be responsible for the incorporation of air bubbles in the liquid egg and plant-based liquid egg analogues (Schramm & Wassmuth, 1994), and their stability during the heat-processing (i.e., cooking of omelets, baking of cakes), as analogized to the stabilizing effects of the surface-active molecules at ALI in the sponge cake batter (Pycarelle, Bosmans, Pareyt, Brijs, & Delcour, 2021; Pycarelle et al., 2020). Hence, the protein and lipid contents (%, d.b.) of the freeze-dried liquid egg, plant-based liquid egg analogues, and foams made thereof were quantified in Table 1. In addition, the protein and lipid recoveries were plotted in Fig. 3A and B to show the molecular migration over HPMC addition and over time.

For the LE sample, whipping reduced the protein-to-lipid ratios, which corresponded to the decreased protein content and the increased lipid content in the LE foams as compared to those in the LE before whipping (Table 1). The foam compositions remained rather constant (Table 1), but their protein and lipid recoveries decreased significantly over time (Fig. 3A and B). These results reflected a phenomenon that the ALI of the LE foams was occupied by an equivalent amount of proteins and lipids, which were drained as a constant fraction of dry matter from the foam phase to the bulk liquid phase over the storage time.

For the plant-based egg analogues, a different molecular transfer behavior to the foam phase was observed. The protein-to-lipid ratios of the foams prepared from the plant-based egg analogues increased significantly from T1 to T3 (Table 1), indicating a gradual displacement of lipids by proteins at the ALI. Over time, proteins were significantly enriched in the 0HPMC, 1HPMC, and 1.5HPMC foams (Table 1), while the protein recoveries decreased significantly (Fig. 3A). It could be concluded that proteins were enriched in these foams as a proportion of the dry matter recovered. In addition, both the lipid content (Table 1) and lipid recoveries (Fig. 3B) in these foams decreased significantly over time. This result suggested that lipids were relatively more abundant at the ALI at the beginning and were gradually discharged from the interfaces to the bulk liquid phase by the surface-active proteins, which was consistent with the previous finding that lipids generally diffused to and adsorbed faster at the ALI than proteins (Damodaran, 2005; Murray, 2007; Pycarelle et al., 2020). Similar behavior was also observed in the sponge cake batter systems (Pycarelle et al., 2020). When the HPMC addition reached 2%, the protein and lipid content decreased significantly from T1 to T3 (Table 1), so did the protein and lipid recoveries (Fig. 3A and B). This observation could be associated with the previous assumption that the dominance of HPMC at the ALI of the 2HPMC foams may hinder the enrichment of proteins and lipids.

Overall, the dry matter was lost from the foams over time, thus the behaviors of molecular enrichment can be known by studying the composition of the remaining dry matter in the foam phase. Foams prepared from the 1.5HPMC plant-based egg analogue had evidently higher protein and lipid contents and recoveries than those prepared from 0HPMC and 1HPMC at every time point, primarily due to the relatively higher retention of proteins and lipids in the former. This contributed to its advantages on foam stability over 0HPMC and 1HPMC (Fig. 1B). It was reported that the existence of HPMC could concentrate the adsorbed proteins by a depletion mechanism due to the limited thermodynamic compatibility between biomacromolecules in the vicinity of the ALI (Pérez, Carrera-Sánchez, Rodríguez-Patino, & Pilosof, 2007); this would generate an osmotic driving force that favored the protein aggregation and increased the diffusion rate (Baeza et al., 2005). On the other hand, 2HPMC retained less protein and lipid composition in the foams and recovered less protein and lipid at T3. It was maybe that

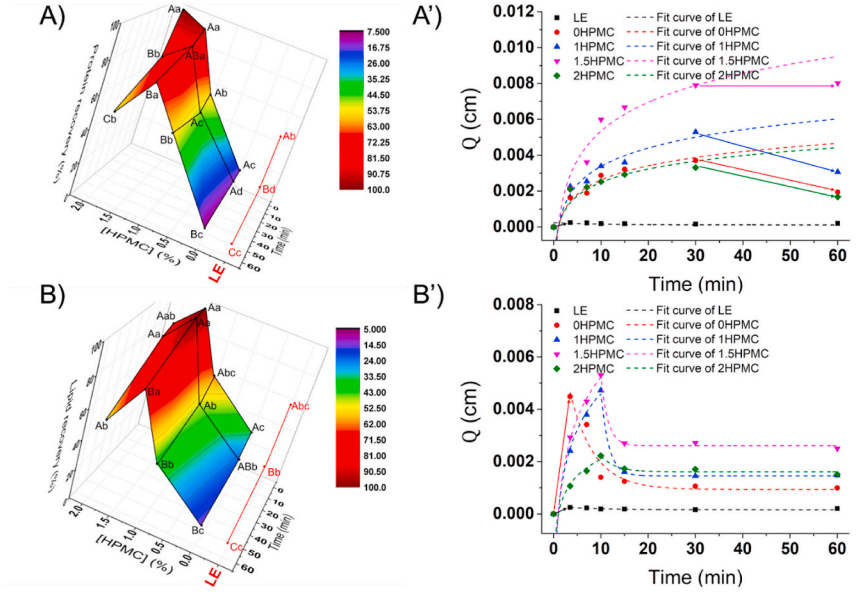

Fig. 3. Recoveries of A) proteins and B) lipids in foams made from liquid egg (LE) and plant-based egg analogues; Mass transfer of A′) proteins and B′) lipids to the foam phase over time. *At each time point, groups with different lowercase letters indicate significant difference ($P < 0.05$) among samples; For the same sample, groups with different capital letters indicate significant difference ($P < 0.05$) among different time point. *0HPMC, 1HPMC, 1.5HPMC, and 2HPMC refer to plant-based liquid egg analogues with addition of 0%, 1%, 1.5%, and 2% HPMC (w/w), respectively.

2% HPMC tended to dominate the ALI and repelled the adsorption of other surface-active components as time went by. HPMC has been mentioned to alter the adsorption of proteins at the ALI depending on its concentration in various systems (e.g., HPMC-soy protein (Martinez et al., 2007); HPMC-whey protein (Pérez et al., 2009); HPMC-β-casein & HPMC-β-lactoglobulin (Arboleya & Wilde, 2005)), mainly due to the higher surface activity of HPMC than these proteins. Moreover, HPMC can improve the partial coalescence of lipid droplets as discussed above. These dose-dependent synergistic/competitive effects may influence the adsorption dynamics in different foam systems, which in turns may affect their rheological and foaming properties.

3.5. Mass transfer of proteins and lipids to liquid egg and plant-based egg analogue foams

From the logarithmic and exponential fitting of the profile *Q* plotted against time, the mass transfer coefficients, RMSEs, and R^2 were obtained as shown in Table 2. The RMSEs of all the molecular transfer models were within 0.05-5.68 × 10^{-4}, demonstrating small deviations between the experimental and predicted values of the molecular transfer after whipping. The R^2 values were greater than 0.90 for all the models, indicating the suitability of using these models in describing the molecular transfer from bulk liquid phase to foam phase during storage. LE foams showed exponential decays in protein and lipid transfer over the storage period (60 min), owing to the decreasing concentrations of protein and lipid in the foam phase. In contrast, the plant-based egg analogues exhibited a time-dependent molecular transfer between the two phases. In the first 30 min, their protein transfer followed a logarithmic behavior, while at T3 (60 min), the protein transfer escaped the logarithmic fitting (Fig. 3A'). The mass transfer coefficients, which represented the mass transfer efficiency and rate (Raffray, Goli, Rivier, Sebastian, & Collignan, 2014), increased from 11.4 to 23.2 μm/min, as the amount of HPMC added increased from 0 to 1.5% (Table 2). The 2HPMC foam had a lower protein transfer coefficient of 10.8 μm/min (Table 2), which might be due to the expelling effect of the HPMC-dominated ALI on protein adsorption.

For the lipid transfer profile, the plant-based egg analogue without HPMC modification (0HPMC) showed a similar exponential decayed pattern as LE over the whole storage period (Fig. 3B'). However, it had a higher lipid transfer rate than LE (-19.7 vs. −13.1 μm/min), indicating the faster discharge of plant-based lipids from ALI than egg lipids. The incorporation of HPMC in the plant-based egg systems retarded the decline phase of lipid transfer-in the first 10 min, lipids transferred to the foam phase logarithmically, while from 10 min to 60 min, lipids were dispelled from the foam phase following an exponential decay trend (Fig. 3B'). The mass transfer coefficients for both the logarithmic increasing phase and the exponential decay phase reached the highest at the addition of 1.5% HPMC ($k_{logarithmic}$ = 22.7 μm/min; $k_{exponential}$ = −67.8 μm/min; Table 2), suggesting the fastest lipid transfer in the 1.5HPMC foams.

Overall, these results reflected that the addition of HPMC up to 1.5% accelerated the mass transfer of proteins and lipids to the foam phase during storage (synergistic phenomena), which might be under conditions of the depletion mechanism (Pérez et al., 2009). Molecular transfers in the 2HPMC foam were slowed down, possibly due to the competitive adsorption that retarded the protein and lipid adsorption at the ALI. The increased mass transfer coefficient of 1.5HPMC foams might contribute to their greater viscoelasticity (Fig. 2D), which is further conducive to their foaming properties (Fig. 1B).

Table 2
Mass transfer of proteins and lipids to the foam phase of liquid egg and plant-based egg analogues.

		Model	Mass transfer coefficient (μm/min)	RMSE (10^{-4})	R^2
Protein mass transfer	LE	Exponential decay	-11.3^{d}	0.05	0.93
	0HPMC	Logarithm	11.4^{c}	2.08	0.97
	1HPMC	Logarithm	14.7^{b}	2.90	0.97
	1.5HPMC	Logarithm	23.2^{a}	5.68	0.96
	2HPMC	Logarithm	10.8^{c}	3.45	0.90
Lipid mass transfer	LE	Exponential decay	-13.1^{c}	0.08	0.90
	0HPMC	Exponential decay	-19.7^{d}	3.52	0.90
	1HPMC	Logarithm	20.0^{a}	0.92	0.99
		Exponential decay	-61.8^{f}	0.08	0.99
	1.5HPMC	Logarithm	22.7^{a}	0.83	0.99
		Exponential decay	-67.8^{g}	0.77	0.99
	2HPMC	Logarithm	9.1^{b}	0.98	0.99
		Exponential decay	-32.5^{e}	0.70	0.90

*Groups with different lowercase letters indicate significant difference ($P < 0.05$) among samples. *LE, 0HPMC, 1HPMC, 1.5HPMC, and 2HPMC refer to liquid egg, and plant-based liquid egg analogues with addition of 0%, 1%, 1.5%, and 2% HPMC (w/w), respectively. *BW, T1, T2, and T3 refer to the diluted plant-based liquid egg analogues before whipping, foams isolated at T1 (3.5 min), T2 (15 min), and T3 (60 min), respectively.

3.6. FTIR analysis of liquid egg, plant-based liquid egg analogues, and foams made thereof

FTIR analysis was conducted to explore the alteration of protein secondary structures at the ALI (Fig. 4). Overall, only minor changes in the secondary structures of proteins were determined, probably because tertiary structural changes were more involved in surface denaturation of protein molecules (Clark, Smith, & Wilson, 1988). Nevertheless, changes in protein secondary structure in response to whipping, storage, and HPMC addition can provide some information on the stability of different foam systems. All samples, including LE and plant-based egg analogues, contained the highest β-sheet fraction. For the LE samples, whipping and storage did not change the composition of protein secondary structure significantly. For the plant-based egg samples, the modification with 1.5% HPMC induced the highest β-sheet content. This indicated the highest HPMC-protein associations, as the increasing β-sheet structure in proteins could present a larger surface area for polysaccharide binding (Chourpa, Ducel, Richard, Dubois, & Boury, 2006; Sow, Chong, Liao, & Yang, 2018). However, a decrease in β-sheets

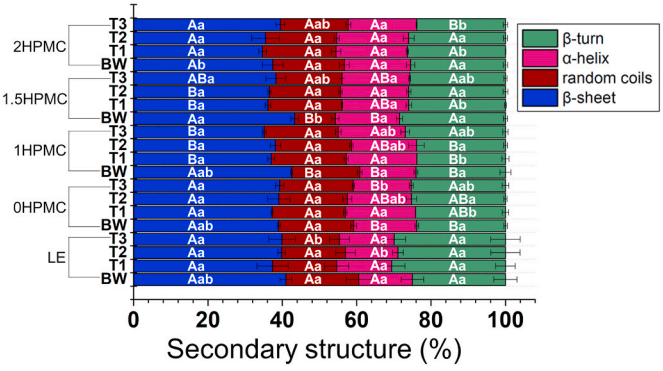

Fig. 4. Protein secondary structure distribution in liquid egg (LE), plant-based egg analogues, and foams made thereof. *At each time point, groups with different lowercase letters indicate significant difference ($P < 0.05$) among samples; For the same sample, groups with different capital letters indicate significant difference ($P < 0.05$) among different time point. *0HPMC, 1HPMC, 1.5HPMC, and 2HPMC refer to plant-based liquid egg analogues with addition of 0%, 1%, 1.5%, and 2% HPMC (w/w), respectively. *BW, T1, T2, and T3 refer to diluted plant-based liquid egg analogues before whipping, foams isolated at T1 (3.5 min), T2 (15 min), and T3 (60 min), respectively.

was reported along with the increase in random coils in the 1HPMC and 1.5HPMC foams, a phenomenon similar to the foamed pepsin sample (Clarkson, Cui, & Darton, 1999). Over time, the reformation of β-sheets occurred only in the 1.5HPMC foams; these recovered β-sheets possessed many non-polar residues that could form a hydrophobic core inside the protein (Bhattacharjee & Biswas, 2009) and then increase the protein hydrophobicity. The hydrophobic proteins could be enriched at the ALI due to their surface activity, forming a viscous film that reduced the surface tension and promoted foam stability (Beverung, Radke, & Blanch, 1999). In addition, there was an increase in α-helix, and a decrease in random coils with increasing HPMC addition in the foams isolated at T3. This meant that HPMC addition may modify the protein conformation through increasing the ordered structure (α-helix) and reducing the random coil structure during the alignment of protein chains under extension of air bubbles. Interestingly, a previous spatially resolved study had reported a greater amount of β-sheets near the bubble surface (Gavin, Verbeek, & Lay, 2019). Our result further supported that the bubbles near the increased β-sheet surface induced by HPMC might grow until they were constrained by the surrounding β-sheets (Gavin et al., 2019), which led to the smaller bubble size in the foams consisting of more HPMC (Fig. 1D).

3.7. Schematic diagram

Based on the results from section 3.1 to section 3.5, a schematic diagram was proposed to elucidate the foaming mechanisms in the plant-based egg analogue systems (Fig. 5). In the 0HPMC system, lipids were gradually expelled from the ALI by the proteins over time (section 3.3), nevertheless, the molecular transfer rates for proteins and lipids were relatively low ($k_{protein}$ = 11.4 μm/min; k_{lipid} = −19.7 μm/min; section 3.4). This resulted in a lower consistency of the 0HPMC foams (section 3.2), and thus an inferior foaming property of the 0HPMC plant-based egg analogue (section 3.1). The modification with 1% HPMC accelerated the transfer of proteins and lipids to the foam ($k_{protein}$ = 14.7 μm/min; k_{lipid} = 20.0 to −61.8 μm/min). In such a system, replacement of lipids by proteins at ALI was still present (section 3.3), meanwhile, partial coalescence of lipid induced by HPMC was observed under CLSM (section 3.7). The synergistic effects between HPMC and the plant-based egg analogue system enhanced the consistency of the 1HPMC foams (section 3.2), which contributed to the improved foaming properties of the 1HPMC plant-based egg analogue (section 3.1). Increasing the HPMC addition to 1.5% increased the transfer rate of proteins and lipids notably ($k_{protein}$ = 23.2 μm/min; k_{lipid} = 22.7 to −67.8 μm/min; section 3.4), mainly due to the volumetric synergy between HPMC-soy protein isolate and HPMC-sunflower oil (section 3.2). The partial coalesced lipid droplets existed in the gap between the gas cells (section 3.7) could further strengthen the cross-linking between the bubbles, thereby improving the viscoelastic (section 3.2) and foaming properties (section 3.1) of the 1.5HPMC plant-based egg analogue. However, with the further increase in HPMC concentration to 2%, HPMC dominated the ALI and exhibited competitive effect on the adsorption of proteins and lipids (section 3.3). This led to a slower protein and lipid transfer to the foam phase ($k_{protein}$ = 10.8 μm/min; k_{lipid} = 9.1 to −32.5 μm/min; section 3.4), reduced synergy and consistency (section 3.2), and reduced foaming stability (section 3.1) of the 2HPMC plant-based liquid egg analogue.

On the whole, the plant-based liquid egg analogue modified with 1.5% HPMC (1.5HPMC) matched the foaming properties of the liquid egg best, owing to its preponderance on the synergistic adsorption of the surface-active components and the cross-linking structure that improved the viscoelastic properties of the foams. In what follows (section 3.8), the plant-based egg analogues with different foaming properties were applied in the omelet dish and sponge cake preparation to evaluate their efficacies in egg replacement.

3.8. Model validation by CLSM

To validate the schematic models proposed above, the two-dimensional CLSM images of the LE, plant-based liquid egg analogues, and foams made thereof are presented in Fig. 6. Additionally, the three-dimensional models of the foams and the volume of each dyed region were obtained based on the stacks of CLSM images (Fig. 6). The green domain represents the dyed proteins and polysaccharides, the red domain represents the dyed oil droplets, and the blue domain represents the dyed cellulose. It was clearly shown that foams prepared from the 0HPMC sample had an oil layer around the bubbles. As the HPMC addition increased to 1.5%, the oil layer at the ALI was gradually replaced by proteins and polysaccharides (increasing volume of blue dye

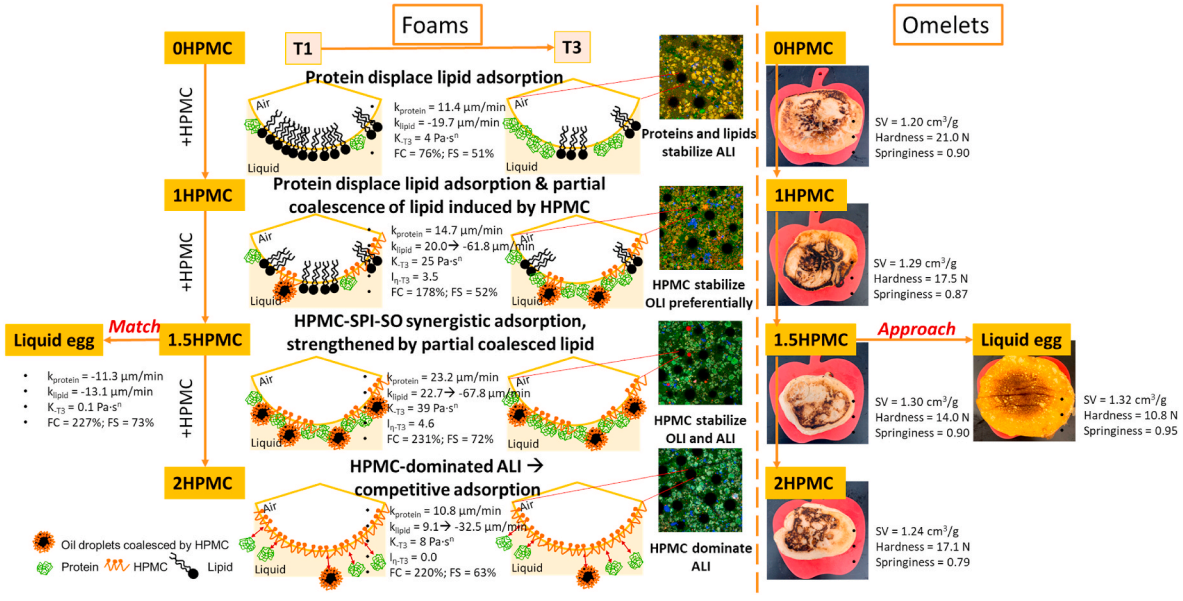

Fig. 5. Schematic diagram elucidating the foaming mechanisms in the plant-based egg analogues modified with different amount of HPMC. *0HPMC, 1HPMC, 1.5HPMC, and 2HPMC refer to plant-based liquid egg analogues with addition of 0%, 1%, 1.5%, and 2% HPMC (w/w), respectively. *FC: foaming capacity, FS: foaming stability, ALI: air-liquid interface, OLI: oil-liquid interface. *T1 and T3 refer to foams isolated at T1 (3.5 min) and T3 (60 min), respectively.

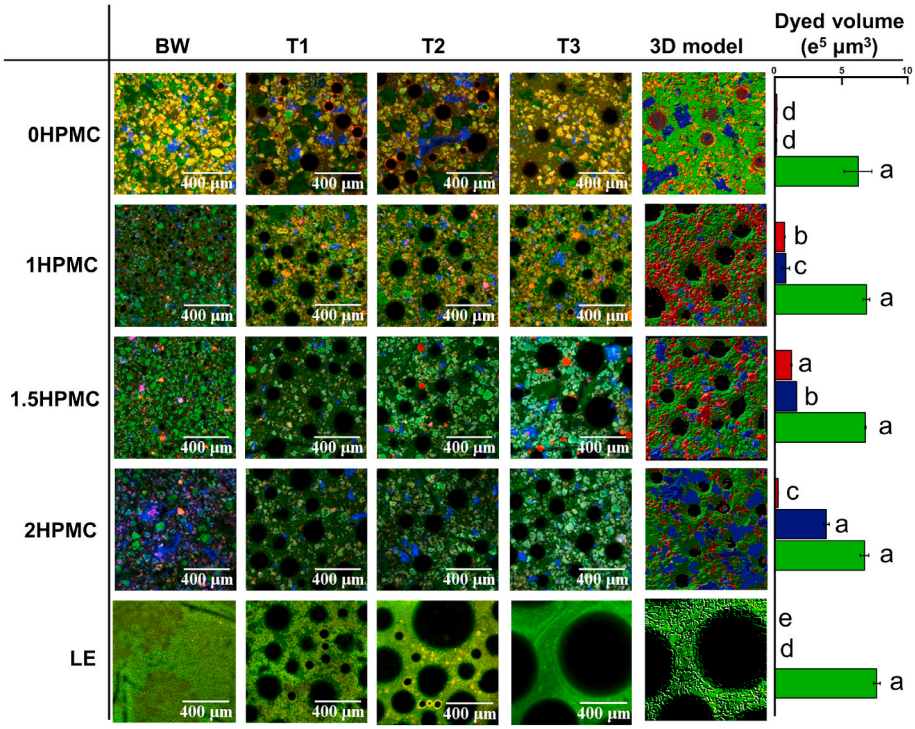

Fig. 6. CLSM images, 3D models, and dyed volume of liquid egg (LE), plant-based egg analogues, and foams made thereof. *0HPMC, 1HPMC, 1.5HPMC, and 2HPMC refer to plant-based liquid egg analogues with addition of 0%, 1%, 1.5%, and 2% HPMC (w/w), respectively. *BW, T1, T2, and T3 refer to diluted plant-based liquid egg analogues before whipping, foams isolated at T1 (3.5 min), T2 (15 min), and T3 (60 min), respectively.

with increasing HPMC addition; Fig. 6), and the oil droplets were partially coalesced under the action of HPMC (increasing volume of red dye with increasing HPMC addition to 1.5%; Fig. 6). These oil droplets tended to penetrate the proteinaceous membrane that enveloped the air bubbles, and then strengthened the interfacial structures of the foams. However, the coalesced oil droplets cannot be observed in the 2HPMC foams (decreasing volume of red dye in 2HPMC; Fig. 6), suggesting that the HPMC-dominated interface may hinder the oil adsorption to the foam phase, as discussed above.

3.9. Application of plant-based liquid egg analogues in omelet dish and sponge cake

The physicochemical properties, including specific volume and textural profile, of the sponge cakes and omelets made from the commercialized liquid egg or the plant-based liquid egg analogues are displayed in Table 3.

The specific volume of the eggless sponge cakes increased significantly as the HPMC addition increased from 0% to 1.5%, which followed a similar trend to the foaming properties. However, the sponge cake made from the 2HPMC plant-based egg analogue could not maintain the specific volume, mainly due to its inferior foaming stability than LE (Fig. 1B). Similar observations were reported on the specific volume of the plant-based omelets. Overall, the foaming properties (section 3.1) were well-related to the specific volumes (Table 3), meaning that both are the measures for evaluating the air incorporated in the eggless products.

Despite the comparable foaming properties of 1.5HPMC to liquid eggs, the eggless sponge cakes were not able to reach the specific volume of that made from liquid egg. The HPMC-modified cakes at 1.5% appeared to approach the specific volume of the authentic cake. Unlike the inferior performance in sponge cakes, substituting eggs with the plant-based egg analogues contained 1% and 1.5% HPMC could provide the omelet dishes with a comparable specific volume. Overall, these

Table 3
Properties of sponge cakes and omelets made from liquid egg or plant-based egg analogues.

Property		Sponge cake					Omelet				
		LE	0HPMC	1HPMC	1.5HPMC	2HPMC	LE	0HPMC	1HPMC	1.5HPMC	2HPMC
Specific volume (cm^3/g)		3.06 ± 0.01^{a}	2.01 ± 0.08^{d}	2.38 ± 0.01^{bc}	2.56 ± 0.17^{b}	2.28 ± 0.06^{c}	1.32 ± 0.03^{a}	1.20 ± 0.03^{c}	1.29 ± 0.03^{ab}	1.30 ± 0.01^{ab}	1.24 ± 0.02^{bc}
Textural profile	Hardness (N)	2.8 ± 0.2^{d}	9.3 ± 1.2^{a}	6.2 ± 0.4^{b}	4.4 ± 0.3^{c}	5.2 ± 0.3^{bc}	10.8 ± 1.8^{a}	21.0 ± 3.7^{a}	17.5 ± 2.0^{b}	14.0 ± 2.9^{b}	17.1 ± 1.7^{b}
	Springiness	0.92 ± 0.01^{a}	0.70 ± 0.02^{b}	0.68 ± 0.07^{b}	0.74 ± 0.05^{b}	0.68 ± 0.07^{b}	0.95 ± 0.05^{a}	0.90 ± 0.09^{a}	0.87 ± 0.06^{ab}	0.90 ± 0.09^{ab}	0.79 ± 0.09^{b}
	Cohesiveness	0.68 ± 0.01^{a}	0.43 ± 0.02^{b}	0.41 ± 0.03^{b}	0.40 ± 0.01^{b}	0.41 ± 0.03^{b}	0.73 ± 0.03^{a}	0.69 ± 0.03^{a}	0.59 ± 0.03^{b}	0.59 ± 0.03^{b}	0.52 ± 0.04^{c}
	Chewiness (N)	1.8 ± 0.1^{b}	2.9 ± 0.4^{a}	1.2 ± 0.2^{c}	1.8 ± 0.2^{b}	1.5 ± 0.1^{bc}	7.3 ± 1.0^{a}	12.8 ± 2.4^{a}	9.7 ± 1.4^{b}	10.7 ± 1.5^{b}	7.0 ± 1.0^{b}
	Resilience	0.29 ± 0.01^{a}	0.14 ± 0.01^{b}	0.13 ± 0.01^{b}	0.13 ± 0.00^{b}	0.13 ± 0.01^{b}	0.36 ± 0.03^{a}	0.39 ± 0.01^{a}	0.20 ± 0.03^{b}	0.18 ± 0.03^{b}	0.15 ± 0.03^{b}

*Groups with different lowercase letters indicate significant difference ($P < 0.05$) among samples. *LE, 0HPMC, 1HPMC, 1.5HPMC, and 2HPMC refer to liquid egg, and plant-based liquid egg analogues with addition of 0%, 1%, 1.5%, and 2% HPMC (w/w), respectively.

promising results indicated that HPMC addition (≤1.5%) could promote the foaming properties of the plant-based egg analogues, and thus improve the quality of the eggless products.

As for the textural profiles, crumb of egg-substituted cakes was harder than that of LE cakes. Also, it was less cohesive and springy (Table 3). HPMC modification reduced the hardness of crumb significantly but had less impact on cohesiveness and springiness. The HPMC-modified eggless omelets were softer, less springy, and less cohesive than that without HPMC modification (0HPMC). These textural properties are most likely related to the greater amount of air trapped in the HPMC-modified crumbs (i.e., higher specific volume), as there was an inverse relationship well-reported between the crumb hardness and volume (Bárcenas & Rosell, 2006). In addition, the presence of HPMC may interact with the amylose in the eggless cakes, thus interfering with the hardening process after baking (Bárcenas & Rosell, 2006). These results agree with the previous studies regarding the conventional bread-making (Bárcenas & Rosell, 2006; Guarda, Rosell, Benedito, & Galotto, 2004), in which the multitudinous impacts of HPMC on amylose leaching and gelation, starch gelatinization, and protein polymerization would account for these phenomena.

4. Conclusion

This study elucidated the foaming mechanisms in the plant-based egg analogues modified by different amounts of HPMC up to 2%. The molecular adsorption at the ALI was described by a two-film theory, with the mass transfer coefficients (k) being related to the rheological and foaming properties of the plant-based egg analogues. The formulation comprising 1.5% HPMC matched the foaming capacity (231% vs. 227%) and foaming stability (72% vs. 73%) of liquid egg. It might be due to the thermodynamic incompatibility between biomacromolecules in the vicinity of the ALI that accelerated the protein and lipid adsorption through depletion mechanism ($k_{protein}$ = 23.2 μm/min, k_{lipid} = 22.7 to −67.8 μm/min). This led to the highest volumetric synergy, consistency, and elasticity in the foams made thereof. Foams containing less HPMC did not have fast molecular adsorption, probably due to less HPMC on inducing synergistic adsorption. Foams contained more HPMC may exhibit HPMC-dominated ALI, which could compete with protein and lipid adsorption and cause reduced foaming properties. It is concluded that HPMC imparted dose-dependent effect on the molecular adsorption at the ALI, which determined the foaming properties of the plant-based egg analogues. In addition, the preponderant foaming properties of the 1.5% HPMC-modified plant-based egg analogue allowed its promising applications in the eggless sponge cake and omelet systems. Further studies should be conducted to explore the interfacial rheological properties of the foams prepared from the plant-based egg analogues. Through the demonstration on foaming mechanisms in a plant-based egg analogue system, this study can provide new insights in optimizing eggless products based on foaming characteristics.

CRediT authorship contribution statement

Zhou Lu: Conceptualization, Methodology, Investigation, Software, Visualization, Validation, Writing-original draft, Writing-review & editing. **Pin-Rou Lee:** Methodology. **Hongshun Yang:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing - review & editing.

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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