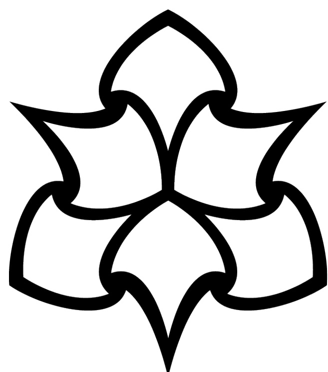


Jiang, X, Shekarforoush, E, Muhammed, MK, Whitehead, KA, Arneborg, N and Risbo, J (2023) Lactic acid bacteria as structural building blocks in non-fat whipping cream analogues. Food Hydrocolloids, 135. p. 108137. ISSN 0268-005X

Downloaded from: https://e-space.mmu.ac.uk/630970/

Version: Published Version

Publisher: Elsevier

DOI: https://doi.org/10.1016/j.foodhyd.2022.108137

Usage rights: Creative Commons: Attribution 4.0

Please cite the published version

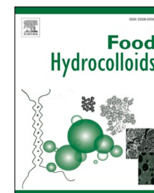
https://e-space.mmu.ac.uk

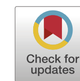
Food Hydrocolloids 135 (2023) 108137

Contents lists available at ScienceDirect

Food Hydrocolloids

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





Lactic acid bacteria as structural building blocks in non-fat whipping cream analogues

Xiaoyi Jiang [a], Elhamalsadat Shekarforoush [a], Musemma Kedir Muhammed [a], Kathryn A. Whitehead [b], Nils Arneborg [a], Jens Risbo [a,*]

[a] University of Copenhagen, Department of Food Science, Rolighedsvej 30, DK-1958, Copenhagen, Denmark
[b] Manchester Metropolitan University, Department of Life Sciences, Chester St, Manchester, M15GD, United Kingdom

ARTICLE INFO

Keywords:
Lactic acid bacteria
Hydrophobicity
Aggregation
Whipping cream
Air-water interface
Structural building blocks

ABSTRACT

Lactic acid bacteria as food ingredients, show the potential of being exploited as structural building blocks in the formulation of colloidal foods such as emulsion and foam. The present work provides approaches to using lactic acid bacteria combined with two components, hydroxypropyl methylcellulose (HPMC) and casein sodium (CS) salt, to fully replace the saturated fat content in whipping cream analogues. By involving both hydrophobic and hydrophilic strains, the whipped cream exhibited comparable overrun (107%) and drainage stability (drainage area 1.4 mm^2) to the commercial dairy whipping cream (30% and 2.7 mm^2, respectively), where the foam stability was greatly affected by the Pickering capability and aggregating properties of the used strains. All the whipped cream displayed solid-like behaviors (G'>G″) and standing properties to different degrees (G' ≈ 30–491 Pa), depending on the strength of bacterial aggregation jointly determined by both the intrinsic surface properties and the influence of added HPMC and CS components. No negative impacts on bacterial viability was found for the added components and the whipping process. The idea of involving edible lactic acid bacteria as fat replacers can thus provide possible alternatives to using nature-derived components as active structural building blocks for colloidal food systems such as whipping cream.

1. Introduction

Food colloidal materials such as emulsions and foams are traditionally constructed using amphiphilic molecules, polymers or small particles as building blocks (Dickinson, 2010). In these structures, the interfaces are initially formed via an emulsification process, which is followed by rapid adsorption of small-molecular surfactants or macromolecules emulsifiers such as caseinate and whey proteins from bovine milk (Ly et al., 2008; Marinova et al., 2009).Moreover, fat globules of saturated fats, due to their solid nature and ability to take part in partial coalescence, are often used as particulate stabilizers to sterically stabilize foam structures either by adsorbing at surfaces through a Pickering effect (Ghosh & Rousseau, 2011; Gupta & Rousseau, 2012), or forming a colloidal network in continuous phase (Peng et al., 2018).

There has been an increasing concern among consumers about high amount of fat in foods due to the high energy density and adverse environmental effects (Shalaby et al., 2013). In order to solve the issues without compromising food structure, texture and mouth feeling, several kinds of alternatives derived from natural resources have emerged as effective fat substitutes in colloidal food materials. For example, oleosomes in plant seeds as naturally-emulsified oil bodies (Nikiforidis, 2019), have been exploited in developing vegan mayonnaise (Romero-guzmán et al., 2020), oleogels (Mert & Vilgis, 2021) and non-fat meat (Anna et al., 2022). Other low-fat foods including ice cream and cake have been produced using less purified fractioned plant components such as micronized cornstarch (Wang et al., 2013) and pea proteins (Feichtinger & Scholten, 2020).

Microorganisms are another type of colloidal materials, either as structural building blocks themselves or as the sources of surface-active compounds. For example, food grade yeasts and non-pathogenic lactic acid bacteria have recently been reported to stabilize emulsion (Firoozmand & Rousseau, 2016), double emulsion (Jiang et al., 2021) and dry foam (Falco et al., 2017; Jiang et al., 2019) via a Pickering effect. The involved structural role of microorganisms as e.g. Pickering particles is greatly dependent on their surface chemical compositions and thereby physicochemical properties such as cell hydrophobicity (Marín

* Corresponding author.
E-mail address: jri@food.ku.dk (J. Risbo).

https://doi.org/10.1016/j.foodhyd.2022.108137
Received 26 May 2022; Received in revised form 1 September 2022; Accepted 9 September 2022
Available online 13 September 2022
0268-005X/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

et al., 1997; Vadillo-Rodríguez et al., 2004), surface charge density (Millsap et al., 1997; Reid et al., 1999) and aggregating properties (Jonsson & Wadström, 1984; Mobili et al., 2009).

Dairy whipping cream is a complex multi-phase system where saturated fat typically comprises approximately 35% of the total concentration (Allen et al., 2008). Such high fat concentration is required to guarantee the whippability of cream during air incorporation, where partially-coalescent fat globules can build solid network anchoring the air bubbles that are initially stabilized by milk proteins and emulsifiers, and thereby the wet foam bears sufficient stiffness and stability to support its own weight and prevent serum drainage (Hunter et al., 2008). Over the recent years, formulations of low-fat whipping cream (fat concentration of 20–30%) have been reported by using a wide range of hydrocolloids such as whey protein concentrates (Salahi & Mohebbi, 2021), modified starches (Athari et al., 2021; Iftikhar & Dutta, 2020), cellulose (Athari et al., 2021) and protein-polysaccharide complexes (Ghribi et al., 2021; Rezvani et al., 2020) as saturated fat replacers. The low-fat whipped cream exhibited comparable physical and textural properties with commercial dairy whipping cream due to the active role of such hydrocolloids in maintaining emulsifying capacity, water retention ability and high viscosity.

The aim of this work was to determine if the comparable structural functionality of solid fat globules, whose action through Pickering effects and partial coalescence can be achieved using edible lactic acid bacteria with selected surface and aggregation properties. In order to explore this, two strains of lactic acid bacteria, *Lactobacillus delbrueckii* subs. *lactis* ATCC 4797 (LBD) and *Lactobacillus crispatus* DSM20584 (LBC) with different surface properties, were combined with two food grade components, casein sodium (CS) and hydroxypropyl methylcellulose (HPMC), to create a series of whipping cream-like suspensions. Following whipping, the physical properties of foam structure including overrun, drainage, bacterial aggregation, rheological properties and microstructures were investigated to evaluate the wider potential of lactic acid bacteria as structural building blocks in foods.

2. Materials and methods

2.1. Materials and chemicals

Glycerol, casein sodium (CS) salt from bovine milk, sodium chloride (NaCl), potassium chloride (KCl), disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4) and FITC (Fluorescein 5(6)-isothiocyanate) were purchased from Sigma-Aldrich, Steinheim, Germany. Low molecular weight hydroxypropyl methylcellulose (HPMC, METHOCEL™ F50 Food Grade Modified Cellulose) was obtained from Dupont de Nemours Inc, Wilmington, DE, USA. *Lactobacillus crispatus* DSM 20584 (LBC) and *Lactobacillus delbrueckii* subs. lactis ATCC 4797 (LBD) were kindly obtained from strain collection of Department of Food Science, University of Copenhagen (Finn Kvist Vogensen, Personal communication). MRS (de Man, Rogosa and Sharpe) broth, MRS agar and atmosphere generation system (AnaeroGen sachets) were bought from Oxoid, Basingstoke, England. The NucRed™ Live 647 ReadyProbes™ Reagent were bought from ThermoFisher Scientific, Molecular Probes, Eugene, OR, USA. All the chemicals were used as received, except for MRS broth and agar which were sterilized in an autoclave (115 °C, 10 min) before use. Sterile MilliQ (MQ) water (18.2 MΩcm at 25 °C) was used in all the experiments.

2.2. Growth of bacteria

Lactobacillus delbrueckii subs. *lactis* ATCC 4797 (LBD) and *Lactobacillus crispatus* DSM20584 (LBC) from the previous stock were inoculated in MRS broth at 37 °C for 24 h, and then culture stocks were prepared by mixing 500 μL culture and 500 μL glycerol (40 v/v%). The prepared culture stocks were stored at −80 °C. For growth of bacteria, 100 μL of frozen stock was anaerobically propagated in 10 mL MRS broth at 37 °C for 24 h. Then, 5 mL and 250 μL of the preculture were anaerobically incubated in 500 and 50 mL MRS broth at 37 °C, respectively, for further investigations. Finally, cells after 24-h growth were collected by centrifugation at 5000×*g* for 10 min at 4 °C, and washed twice with sterile MQ water. Cell suspension corresponding to 250 μL preculture in 50 mL broth was used for the characterization of bacterial cells, while suspension corresponding to 5 mL preculture in 500 mL MRS broth produced 1.5 g wet pellets for both strains, which was used for the preparation of whipping cream.

2.3. Characterization of bacteria

2.3.1. Surface charge

The zeta potential of LBD and LBC was measured by a zeta sizer (Malvern Zetasizer, Nano ZSP, UK) at 25 °C using MQ water as background electrolyte solution. For the measurement, pellets corresponding to 250 μL preculture in 50 mL MRS broth were re-suspended in 50 mL MQ water, which was further diluted 10 times with MQ water. Then, 1 mL of the diluted suspension was injected into the capillary cell using a disposable syringe. Before each measurement, the capillary cell was rinsed subsequently with ethanol, MQ water and the sample.

2.3.2. Water contact angle measurement

To investigate the wettability of LBD and LBC, the water contact angles, θ of bacterial lawns were measured following a previously-reported protocol (Jiang et al., 2021) with minor modifications. First, cell pellets corresponding to 250 μL preculture in 50 mL MRS broth were re-suspended in 25 mL MQ water, which was filter though a 0.45 μm (pore size) polyvinylidene difluoride membrane assisted by negative pressure until the deposition of a thick bacterial lawn. The bacterial-deposited membrane was fixed onto a microscopic glass slide using double-sided tapes and air-dried in a clean fume hood for 70–90 min, which allowed the formation of plateau contact angle (Bellon--Fontaine et al., 1996). The sessile drop measurement of water contact angle was performed at room temperature by dispensing a water droplet of 3 μL on the bacterial lawn using optical contact angle measuring and contour analysis system (OCA 25, Dataphysics Instruments, Stuttgart, Germany). For each sample, three membranes were prepared and at least 5 drops were dispensed on the same membrane in different dried areas.

2.3.3. Microbial adhesion to hexadecane (MATH)

The hydrophobicity of bacteria was complementarily evaluated using MATH according to a previous protocol (Jiang et al., 2021). Briefly, bacteria are suspended in 10 mM KH_2PO_4 solution and the cell suspension was adjusted to an optical density (OD) value of 0.8 at 600 nm (SpectraMax i3x, Molecular Devices LLC, USA), corresponding to a cell concentration of 1.0×10^8 colony forming units (CFU)/mL. Then, 250 μL cell suspension was mixed with 42 μL hexadecane in an Eppendorf tube, which was incubated at room temperature for 10 min before being vigorously vortexed at 3000 rpm for 90 s with a1-min pause following every 30-s mixing. After vortexing, the mixture was left to stand for 15 min to allow phase separation and then 200 μL of the lower aqueous phase was transferred to a 96-well plate to measure the OD value at 600 nm. The percentage of MATH was obtained by;

$$\%\ \text{MATH} = \left(1 - \frac{A_1}{A_0}\right) \times 100\% \tag{1}$$

where A_0 is the initial OD600 of the bacterial suspension and A_1 is the OD600 value of the lower aqueous phase after mixing and phase separation.

2.3.4. Aggregation assay

The ability of bacteria to aggregate ("autoaggregation" as microbiological term) was investigated with respect to their sedimentation over

time based on a previously-reported protocol (Polak-Berecka et al., 2014) with minor modifications. First, cell pellets corresponding to 250 μL preculture in 50 mL MRS broth were re-suspended in either MQ water or NaCl (50 mM). The OD of the resultant cell suspensions at 600 nm was adjusted to 1.0, corresponding to a cell concentration of 2 × 10^8 CFU/mL. The suspensions were left to stand at room temperature for 4 h, and the upper part of the suspensions (around 0.5 cm below the liquid level) was transferred to measure the OD at 600 nm without disturbing the lower part of the suspension. The bacterial sedimentation termed as the aggregation coefficient (AC) was calculated based on the equation;

$$AC = \left(1 - \frac{A_{4h}}{A_i}\right) \times 100 \tag{2}$$

where A_i and A_{4h} represent the OD values at initial time and after 4 h, respectively.

2.4. Preparation of whipping and whipped cream

To prepare the aqueous phase used for whipping cream, the powder of HPMC (1.5 w/v%) and CS (1.5 w/v%) were, either individually or after blending, added to MQ water. The mixtures were continuously stirred for at least 12 h at 4 °C to allow complete dissolution of HPMC and CS. Before use, the solution was passed through a 0.22 μm-pore-size cellulose membrane and the clear solution was used within 24 h after preparation.

Bacterial pellets collected from 5 mL preculture in 500 mL broth were re-suspended in 2.5 mL of the aforementioned solutions and gently stirred at 4 °C for 15 min to obtain homogeneous bacterial suspensions, which generated 4 mL fresh whipping cream. The fresh whipping cream was aged by storing at 4 °C for at least 12 h before whipping. For the whipping process, the aged whipping cream was transferred to a 10-mL beaker and whipped using a kitchen milk frother (Ariete, Mod.623, Italy) at the speed of 11000 rpm for 90 s, which consisted of continuous mixing for the first 40 s and 10 s intervals for the final 50 s. The whipped cream was stored at 4 °C until investigations within 30 min. The formulations of produced whipping cream are summarized (Table 1). A commercial dairy whipping cream (fat content 38%) was also prepared for the investigation of whipping properties including overrun and drainage.

2.5. Whipping properties

2.5.1. Overrun measurement

The overrun of whipping cream was investigated according to a standard method (Bruhn & Bruhn, 1988) with minor modifications. Briefly, the identical volume of unwhipped and whipped cream was transferred to pre-weighed 1.5-mL Eppendorf tubes, and these were weighed. The overrun referring to the amount of incorporated air was calculated based on the following equation;

$$Overrun = \left(\frac{m_1 - m_2}{m_1}\right) \times 100 \tag{3}$$

Table 1
Formulations of whipping cream produced using two lactic acid bacteria strains, LBC and LBD.

Strain	HPMC, w/w%	CS, w/w%	Code
LBC (38 w/w%[a])	0.9	0	LBC-HPMC
	0	0.9	LBC-CS
	0.9	0.9	LBC-HPMC-CS
LBD (38 w/w%[a])	0.9	0	LBD-HPMC
	0	0.9	LBD-CS
	0.9	0.9	LBD-HPMC-CS

[a] The concentrations of bacteria were estimated based on the volume of wet pellets.

where m_1 and m_2 represent the weight of unwhipped and whipped cream, respectively, with identical volumes.

2.5.2. Evaluation of drainage

The foam stability of whipped cream was investigated with respect to the drainage of aqueous phase using an in-house filter paper method. After whipping, 0.5 g of whipped cream was transferred onto a filter paper (Whatman filter, grade 4, UK), which was placed in a closed chamber at room temperature to prevent water evaporation. After 30 min, the diameter of the circular wet area on the filter papers was measured using a ruler. The total drainage of samples within 30 min was represented as the wet area (mm^2) on each filter paper.

2.6. Rheological properties of whipped cream

The viscoelasticity of whipped cream was measured at 25 °C using Discovery Hybrid Rheometer (DHR-2, TA instruments, New Castle, DE) equipped with a serrated parallel plate measuring geometry (25 mm diameter and 0.5 mm gap). In the dynamic viscoelastic measurements, the linear viscoelastic range (LVR) was firstly determined by performing the strain sweep test in a range of 0.1%–100% at a fixed frequency of 1 Hz, to prevent the degradation of microstructure. A constant strain level of 0.5% was found within the LVR of all samples, which was further used to conduct the frequency sweep test at a range from 0.1 to 10 Hz. In all the measurements, samples were freshly-whipped and measured within 5 min. The results were presented as the storage modulus (G'), loss modulus (G'') and loss tangent (tanδ) at a frequency of 1 Hz obtained from the spectra of duplicated frequency sweep tests.

2.7. Size of bacterial aggregates

The size of bacterial aggregates in terms of size distribution and the volume weighted mean size D(4,3) was assessed for fresh and aged whipping cream, as well as for whipped cream using a laser diffraction particle size analyzer (Mastersizer 3000, Malvern Instruments, Workshire, UK) at 25 °C. The samples were slowly added to a dispersion unit filled with distilled water under mild stirring at a velocity of 800 rpm, in order to not break the aggregated bacteria, which was followed by pumping them into the optical chamber for measurement. The obscuration range was between 5% and 13%. The refractive index and the absorption index were set to 1.39 and 0.01, respectively. Before the measurement of whipped cream, samples were placed in a vacuum chamber for 15 s to remove the air bubbles.

2.8. Confocal laser scanning microscopy

The microstructure of whipped cream was investigated using a confocal laser scanning microscope (Point Scanning Confocal and 2-photon microscope SP5-X MP UV, Leica Microsystems, Germany). For staining bacteria, pellets corresponding to 5 mL preculture in 500 mL broth were re-suspended 4 mL MQ water. To that suspension, 8 drops of NucRed™ Live 647 ReadyProbes™ Reagent were added, which was followed by 15-min incubation in the darkness at room temperature. The stained bacteria were washed twice with MQ water by centrifugation at 5000 rpm for 5 min, and used for the preparation of whipping cream. For the staining of CS, 10 μL FITC solution (0.0045 w/v% in acetone) was placed onto a standard glass slide, which was air-dried in darkness for 5 min before gently mixing with 30 μL of sample. The excitation wavelengths of NucRed and FITC were 638 and 495 nm, respectively. All the samples were observed at 200× magnification within 10 min after sample preparation to minimize moisture evaporation. The line average of scanning was set to 8 and the final resolution of the images was 1.52 μm/1.52 μm for X and Y dimension, respectively. The obtained images were further used to analyze the air bubble sizes by manually measuring the diameters of individual bubbles using Leica Microsystems LAS AL

lite software (Germany). The images presented were the most representative ones, and the reported air bubble sizes were analyzed based on a minimum count of 100 bubbles.

2.9. Bacterial viability

The viability of bacteria in whipped cream was quantified by using a plate-counting method. Briefly, bacteria collected after centrifugation or from the whipped cream were transferred into PBS buffer (pH 7.4) using an inoculating loop, where the final OD of the resultant cell suspension at 600 nm was adjusted to 1.0 for all the samples. Serial dilutions (from 10^1 to 10^6) were prepared and 30 μL of each dilution was evenly dispensed in 5–6 drops on one quarter of the MRS agar plate in duplicate. After anaerobically incubating for 48 h at 37 °C, dilutions with 30–300 colonies were selected for counting, and the viable count was expressed as CFU/mL.

2.10. Statistical analysis

All the reported data are the averages of at least duplicated experiments, and the results are presented as average ± standard error. The one-way analysis of variance (ANOVA) was carried out using Fisher's LSD test to detect whether the differences between samples were statistically significant ($P < 0.05$). Analyses were performed using the computer Software OriginPro 2020 (OriginLab Corporation, Northampton, USA).

3. Results

3.1. Surface properties of bacteria

The surface hydrophobicity of bacteria is traditionally investigated using a set of methods including contact angle measurement and MATH, where the former measures cell wettability using the contact angle (θ) of a water droplet resting on a semi-dry bacterial lawn (van Loosdrecht et al., 1987), and the latter measures the degree of bacterial adhesion to non-polar hexadecane droplets through a Pickering effect (Bellon-Fontaine et al., 1996).

Surface properties of bacteria including charge density, hydrophobicity and aggregation ability were determined (Table 2). Regarding the hydrophobicity, LBC exhibited higher values of θ (61.3° ± 4.1°) and MATH (66.4% ± 4.6%) as compared to 33.0° ± 3.6° and 11.3% ± 2.7% for LBD. The adsorption energy of Pickering particles at interfaces (Binks, 2002; Rayner et al., 2014) is proportional to the term $(1-|\cos\theta|)^2$ and the increased contact angle of LBC resulted in one order of magnitude larger value of this term as compared to LBD.

Even though the zeta potential of LBC was slightly more negative than that of LBD, their surfaces were both negatively-charged, indicating that the electrostatic repulsions between cells will play a role in media of low ionic strength. Indeed, the aggregation of LBC and LBD was dramatically promoted when salt was present due to the screening of electrostatic effects, as seen from the higher Ac (NaCl) than Ac (MQ). The difference in bacterial aggregation (within 4 h) was not so evident for two strains, except that compared with LBD, the aggregation of LBC was slower in MQ but became faster in media with high ionic strength.

Table 2
Surface properties in terms of hydrophobicity, charge density and aggregating property of LBC and LBD bacteria.

Strain	θ, degree	$(1-\|\cos\theta\|)^2$	MATH (%)	Zeta potential in MQ (mV)	Ac (MQ)	Ac (NaCl)
LBC	61.3 ± 4.1^a	0.3 ± 0.1^a	66.4 ± 4.6^a	−32.6 ± 1.3^a	0.4 ± 0.0^a	0.9 ± 0.0^a
LBD	33.0 ± 3.6^b	0.03 ± 0.01^b	11.3 ± 2.7^b	−24.6 ± 1.7^b	0.5 ± 0.1^a	0.8 ± 0.0^a

Values are represented as mean values resulted from duplicated experiments. Within each column, different letters indicate significant differences ($P < 0.05$) from a given sample.

3.2. Whipping properties

The whipping process was conducted for a small whipping volume (4 mL) and the whipped samples were investigated in terms of the overrun and drainage (Fig. 1). Besides the formulations listed (Table 1), the control samples containing only bacteria and without bacteria were also whipped. However, air incorporation was not observed for samples containing only bacteria without HPMC and CS, regardless of the used strains. This means that air incorporation was mainly attributed to the use of HPMC and CS other than bacteria due to faster adsorption of molecular surfactants than micron-sized bacterial cells. Therefore, the whipping properties of suspensions without HPMC and CS are omitted and will be commented further.

To measure the overrun, multiple transfer steps of samples were involved and therefore phase separation might have occurred in some unstable samples, leading to the measurement of only dry foam or serum phase. However, this was not a problem for stable samples that were able to retain serum phase. First, all the samples displayed either comparable or higher overrun than the commercial dairy whipping cream (Fig. 1A). As both HPMC and CS are surface active (Dickinson, 2012; Pérez et al., 2008), all the controls without bacteria displayed initially high overrun, but rapidly collapsed within 10 min, as further reflected by their high drainage (Fig. 1B). In contrast, samples containing bacteria generally created more solid-like foams that could stand and support their own weights. Among all the formulations, samples containing HPMC gave higher air incorporation (approx. 107% and 82% for HPMC and HPMC-CS, respectively) than samples with only CS (33%), independent on bacterial strain, LBC and LBD.

The drainage of samples evaluated using a customized filter paper method was determined (Fig. 1B). Under the conditions used in the assay, the drainage was driven by both gravity and the capillary forces of the filter paper, resulting in the formation of wet area which was representative of the volume of the drained serum phase. Unlike overrun, the drainage was systematically dependent on the involved strain, with LBD samples giving nearly twice the drainage of the LBC samples. The drainage of formulations followed a systematic order: CS > HPMC > HPMC-CS. The LBC formulations of HPMC and HPMC-CS obtained even lower drainage (2.1 and 1.4 mm^2, respectively) than the dairy whipping cream (2.7 mm^2). In view of this, the drainage stability was seemingly maintained by the viscosity of samples (see Fig. S1), yet at the same time, affected by the surface properties of bacteria, governing their capability to hold liquid inside the structure via capillary effects.

3.3. Viscoelastic behavior of whipped creams

The spectra of frequency sweep tests demonstrated that the rheological properties were almost independent on frequency in the range of 0.1–10 Hz (Fig. S2). The viscoelastic parameters including G′, G″ and tanδ at a frequency of 1 Hz are thus shown as representative data (Fig. 2). It was found that all the samples exhibited higher values of G′ than G″ as well as loss tangents lower than 1, suggesting a more solid than liquid character possessed by the whipped samples. Specifically, formulations with LBC always showed much higher G′ values of a factor of 3–15 than LBD samples, depending on the different formulations. Despite the higher G′ of LBC samples, they interestingly displayed higher tanδ as well, thus demonstrating that samples containing LBD were less stiff but showed a more solid character than the LBC samples in terms of low damping effect. Furthermore, the two components HPMC and CS seemingly showed opposite effects on the stiffness of samples containing LBC and LBD. For LBC samples, formulations involving CS (CS and HPMC-CS) normally resulted in higher G′, low damping and thus a stronger solid-like property, whereas HPMC tended to work better increasing the stiffness of LBD samples, as seen from the higher G' values

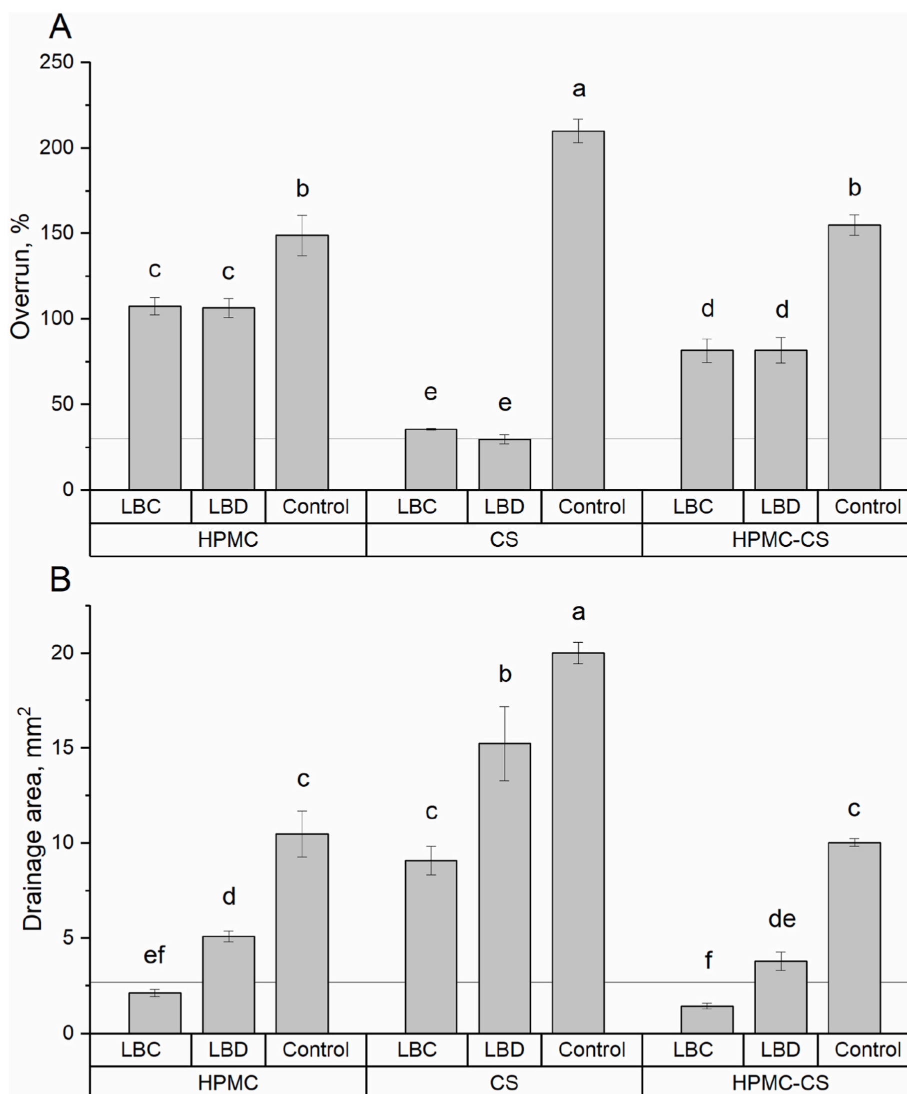


Fig. 1. Whipping properties in terms of overrun (A) and drainage (B) of HPMC, CS and HPMC-CS formulations containing LBC, LBD bacteria and control samples without bacteria. Reference lines represent corresponding properties of commercial dairy whipping cream. Error bars represent standard errors (n = 2). Different letters indicate significant differences ($P < 0.05$) from a given sample.

of HPMC and HPMC-CS samples than CS sample combining with LBD.

3.4. Bacterial aggregation in whipping cream

Static light scattering was used to analyze the degree of bacterial aggregation induced by the aging and whipping processes, which potentially destabilized freely-suspended cells and enhanced aggregation. The size distribution of bacterial aggregates was measured for fresh, aged and whipped samples of different formulations, as well as control samples containing only bacteria (Fig. 3).

Bacterial aggregation in the fresh control samples without HPMC and CS was comparable for LBC and LBD, in agreement with their negligible difference measured in aggregation assay. However, the aggregation of LBC was more promoted after overnight storage than LBD, which only showed negligible changes compared with the fresh sample (Fig. 3G and H). Likewise, in all the formulated samples, both aging and subsequent whipping processes induced increased aggregation of LBC when compared to the LBD, even though LBD tended to show relatively strong aggregation in the fresh samples. Compared to the fresh samples with only HPMC consisting of predominantly free cells than aggregates (Fig. 3A and B), the involvement of CS component facilitated the aggregation of both LBC and LBD in all the fresh samples, which was particularly pronounced for LBC samples (Fig. 3C–F). Interestingly, the whipping process seemed to produce even more free cells and smaller aggregates for LBD samples, which might be explained by either the liberated cells that originally adsorbed at air-water interface after degassing, or the breakdown of weakly-bound bacterial aggregates.

The effects of the different components HPMC and CS on bacterial aggregation in whipped samples were further demonstrated in terms of mean diameter D(4,3) values of aggregates (Fig. 4). First, the overall bacterial aggregation after the whipping process was much stronger for hydrophobic LBC (61.7–94.1 μm) than hydrophilic LBD (8.9–26.3 μm), regardless of formulations. Within all the LBC samples, it was clear that CS-containing samples (CS and HPMC-CS) formed larger bacterial aggregates than samples with only HPMC, in line with the observation from size distribution of LBC samples. However, this was not the case for LBD samples, where CS seemed to show an oppositely lowering effect on bacterial aggregation, as seen from the decreasing aggregate size in the order of HPMC > HPMC-CS > CS. Therefore, the aggregation of LBC in the whipped samples was primarily enhanced by the CS component, while for LBD samples HPMC was on the other hand more capable of promoting bacterial aggregation.

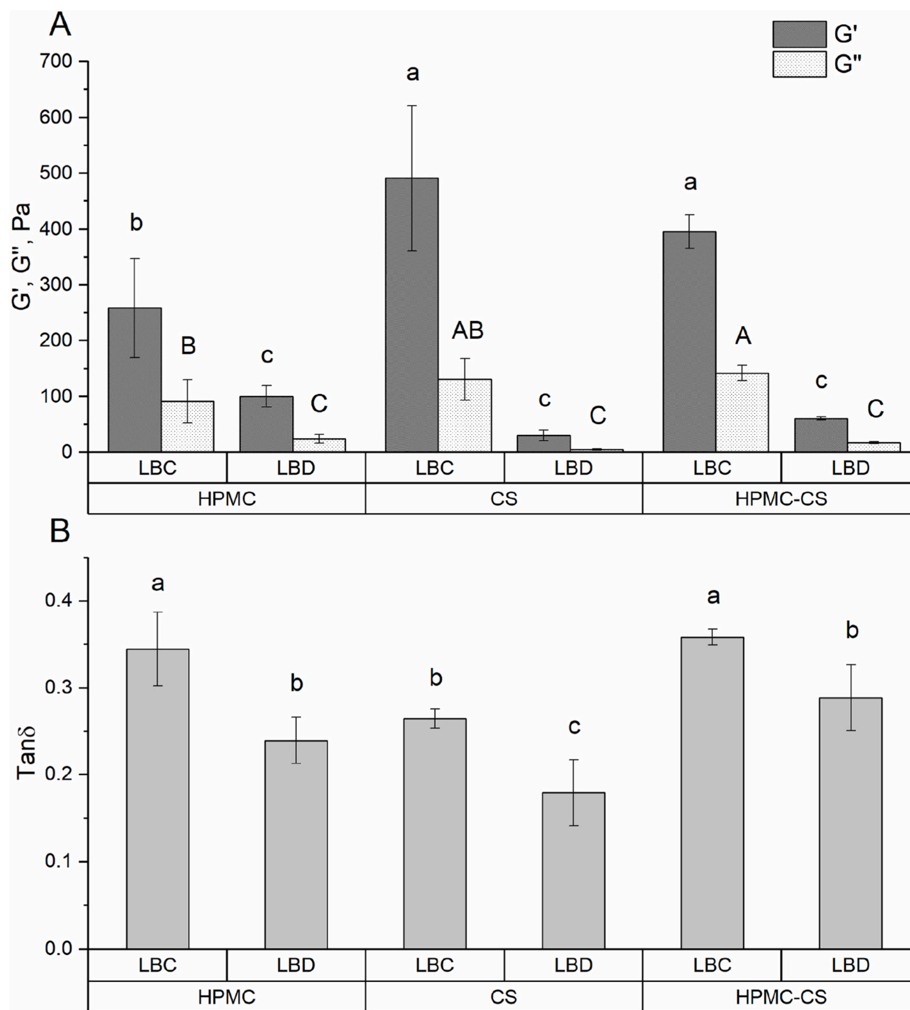


Fig. 2. Dynamic rheological parameters (frequency at 1 Hz and 25 °C) including storage modulus (G′), loss modulus (G″) (A) and loss tangent (B) of whipped cream produced using HPMC, CS and HPMC-CS formulations containing LBC and LBD bacteria, respectively. Error bars represent standard errors (n = 2). Lower case letters (a–c) indicate statistical grouping for G′ data whereas upper case letters (A–C) are used for *G*″ data. Different letters indicate significant differences (P < 0.05) from a given sample.

3.5. Micro- and macro-structure of whipped cream

The CLSM micrographs of the whipped structure are shown (Fig. 5), where the location of bacteria is highlighted using NucRed dye. For control sample without bacteria, rapid bubble coalescence and structural collapse were observed during the microscopic observation and therefore this sample was excluded for the investigation due to the instability. Likewise, the low stability of LBD-CS sample also led to some degree of bubble coalescence, resulting in the capture of multiple irregular bubbles under merging, which correlated well with their low overrun and drainage stability (Fig. 5D).

The biggest difference between LBC and LBD samples was the degree of bacterial adsorption on air bubbles. In contrast to the hydrophilic LBD that was seldom adsorbed to the interface of the air bubbles, the more hydrophobic LBC exhibited almost full coverage at air-water interface, indicating a stronger Pickering capability possessed by LBC than LBD, which is also consistent with the higher water contact angle and MATH values of LBC. As a consequence of strong adsorption, a lower fluorescence intensity of LBC was observed in the aqueous phase, where bacteria were non-adsorbed and tended to aggregate. Hence, a strong Pickering effect can be accompanied with the depleted number of bacteria in the rest of the system. Interestingly, dark rings without bacteria were observed surrounding the air bubbles in LBD samples, and the rings were much thicker in HPMC-containing samples (Fig. 5B and F). This is an indication of depletion flocculation induced by the polymeric component HPMC, creating areas of only polymer solution that completely wetted air bubbles with the absence of bacteria. In this case, the acting polymer(s) might predominantly be HPMC and partially be the exopolysaccharides secreted by LBD (Bancalari et al., 2022). However, the same phenomenon was not observed in LBC samples, whereas multiple dark holes were present independent on the addition of HPMC, and air bubbles were also better wetted by bacteria than the dark holes.

The upper right inserts show macroscopic photos of whipped cream. Samples with LBC clearly showed better standing ability than LBD samples, complying with the more solid-like behavior of LBC samples assessed by rheological analysis. Among all the LBC samples, HPMC-CS formulation displayed a strongest standing property, as also confirmed by its high G' value. For LBD samples, HPMC-containing formulations generally led to whipped cream of higher standing ability.

3.6. Air bubble sizes

The normalized size distributions of air bubbles analyzed from the CLSM micrographs are displayed (Fig. 6). For LBC samples, compared with a broad size range given by formulation containing only HPMC, a narrower and smaller size distribution was obtained for CS-containing formulations (Fig. 6C and E), indicating the important role of CS in creating small size of air bubbles in LBC samples. On the contrary, LBD samples tended to display a narrower and smaller distribution when HPMC was present in the systems (Fig. 6B and F), with combined formulation HPMC-CS leading to the smallest size distribution. Hence, compared with the importance of CS in LBC samples, HPMC rendered the higher stability of air bubbles in LBD samples. These findings were well correlated with the results from size measurement of bacterial

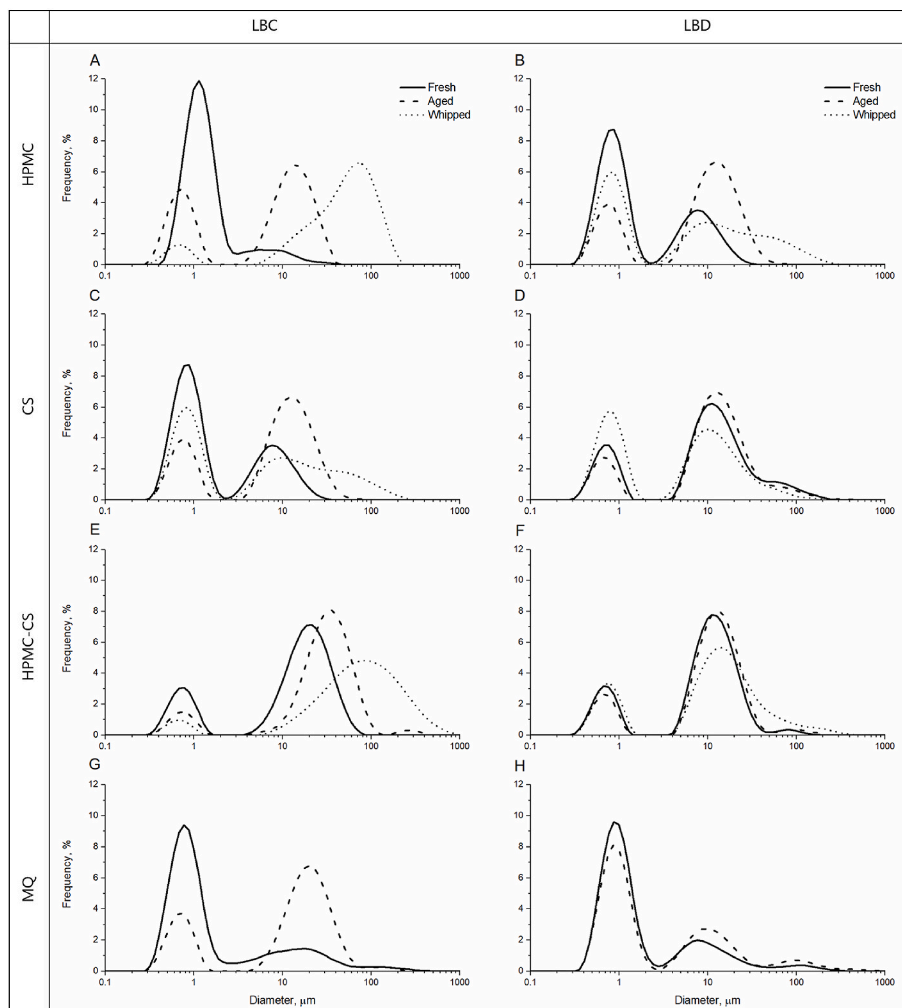


Fig. 3. Size distributions of bacterial aggregates in the fresh, aged and whipped whipping creams measured by static light scattering. The investigated samples included formulations of HPMC (A, B), CS (C, D), HPMC-CS (E, F) and MQ water (G, H) containing LBC (A, C, E, G) and LBD (B, D, F, H) bacteria, respectively. Results are obtained from duplicated measurements.

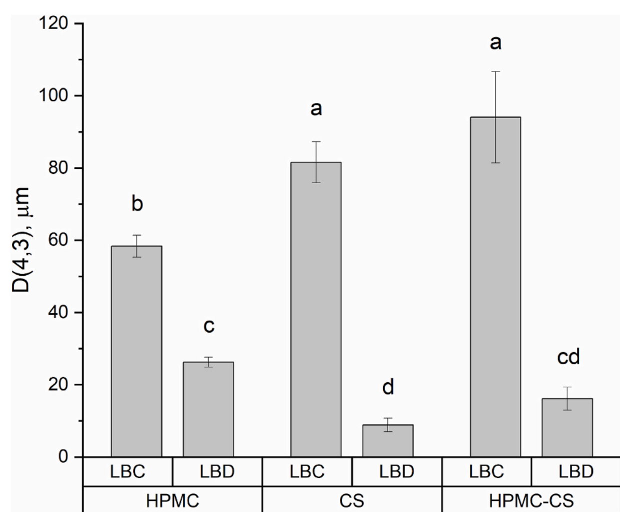


Fig. 4. Mean diameters of bacterial aggregates in terms of D(4,3) values of whipped cream formulated using HPMC, CS, HPMC-CS containing LBC and LBD bacteria, respectively. Error bars represent standard errors (n = 2). Different letters indicate significant differences ($P < 0.05$) from a given sample.

aggregates for all the whipped cream, possibly suggesting the enhancement of air bubble stability by stronger bacterial aggregation via forming steric barriers against bubble coalescence.

3.7. Bacteria – protein interactions

In order to gain insight into the location of CS and possible interactions between CS and bacteria, the CS component in both LBC and LBD samples of HPMC-CS formulation was stained using FITC dye of green fluorescence (Fig. 7A and D). Since there are free amines present on bacterial surface (Leone et al., 2006; Ojeda et al., 2008), a consideration was that FITC staining CS might also label the surface proteins of bacterial cells. However, air bubbles in LBC samples emitting strong red fluorescence of adsorbed LBC were found dark without green fluorescence from CS (Fig. 7A and B), indicating that FITC did not stain the LBC bacteria. For LBD, this phenomenon was less clear and CS also occasionally co-localized with bacteria (Fig. 7D and E), possibly suggesting either the little adsorption of CS on air bubbles or weak staining of LBD by FITC dye. In the combined channel of bacteria and CS, it was clear for LBC sample that an enhanced signal of CS was only present where LBC bacteria were also abundant (Fig. 7C), while multiple dark holes without red signal of bacteria emitted weaker green fluorescence from CS, which indicated almost the only co-localization of CS and aggregated LBC in the system. By comparison, CS in the LBD sample exhibited not only the co-localization with LBD bacteria, but also CS-alone regions of strong

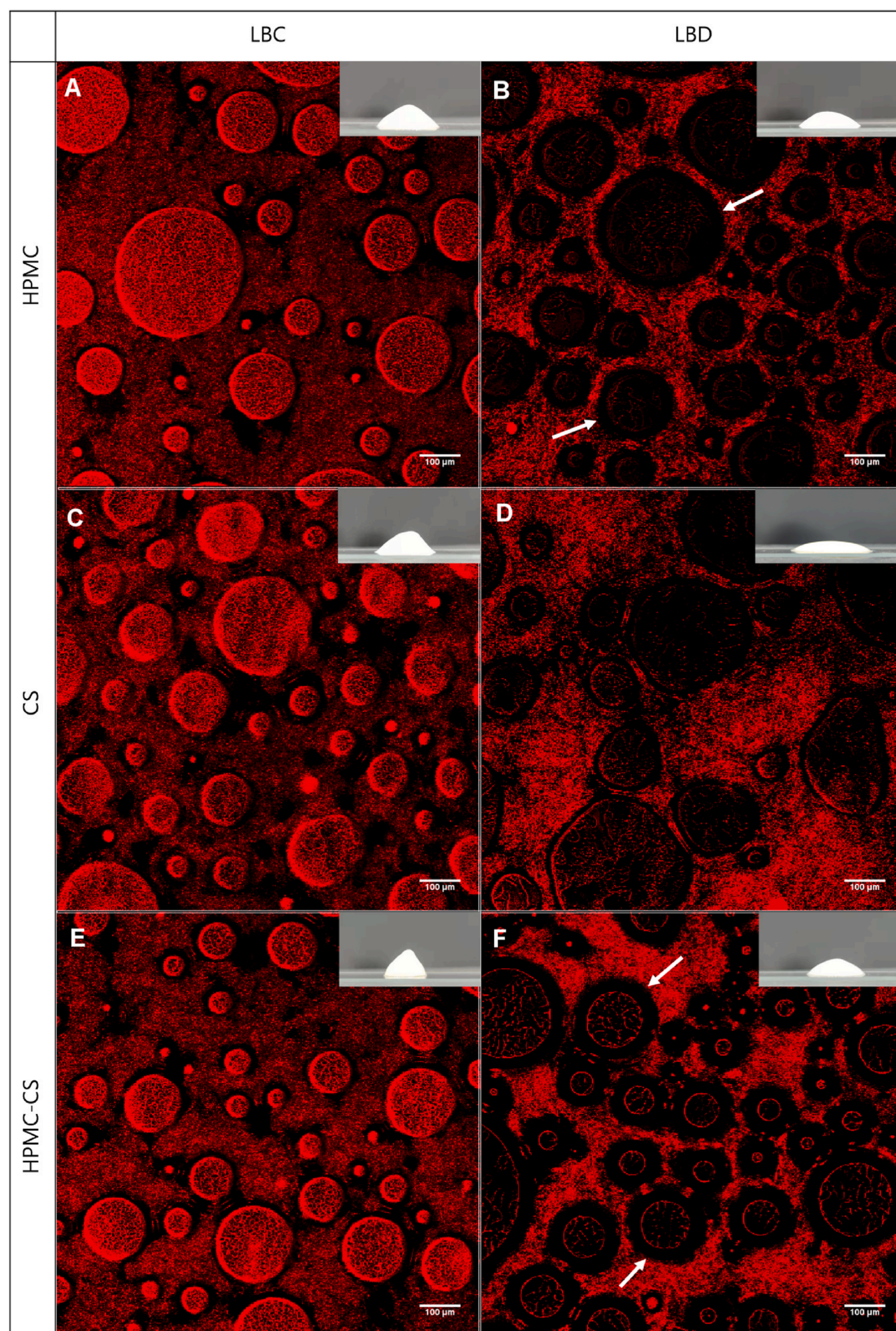


Fig. 5. CLSM micrographs and macroscopic photos of whipped cream with HPMC (A, B), CS (C, D) and HPMC-CS (E, F) formulations containing LBC (A, C, E) and LBD (B, D, F) bacteria, respectively. Non-fluorescent rings surrounding air bubbles (white arrows) were observed in LBD samples containing HPMC but not in LBC samples, indicating the clear depletion flocculation of LBD induced by HPMC polymers. The scale bars represent 100 μm.

green fluorescence that were located in the proximity of air-water interface, indicating the surface activity of CS component and a possibly lower affinity of CS to LBD than to LBC. The LBC and LBD bacteria showed different degree of partitioning into CS phase, but they were neither surface-active at the CS-HPMC interface nor partitioning into the HPMC phase as observed as lack of enhanced red signal from the cells. Instead, bacteria displayed overall stronger surface activity at air-water interface as seen from their adsorption on the air bubbles, which was in turn much clearer for the hydrophobic LBC than hydrophilic LBD.

3.8. Viability of bacteria in whipped cream

The culturability of bacteria in whipped cream determined using plate-counting method was demonstrated (Fig. 8). The complementary results of viability in terms of cell membrane integrity was also obtained by using fluorescence microscopy using LIVE/DEAD viability kit (Fig. S3). Compared with the CFU/mL of control LBC samples directly harvested from centrifugation (7.2 log), the culturability of LBC in all the whipped samples showed around a one-log decrease, regardless of the formulations, whereas the CFU/mL reduction induced by aging and whipping processes was not observed for the more hydrophilic LBD bacteria. However, a contradiction was found with fluorescence

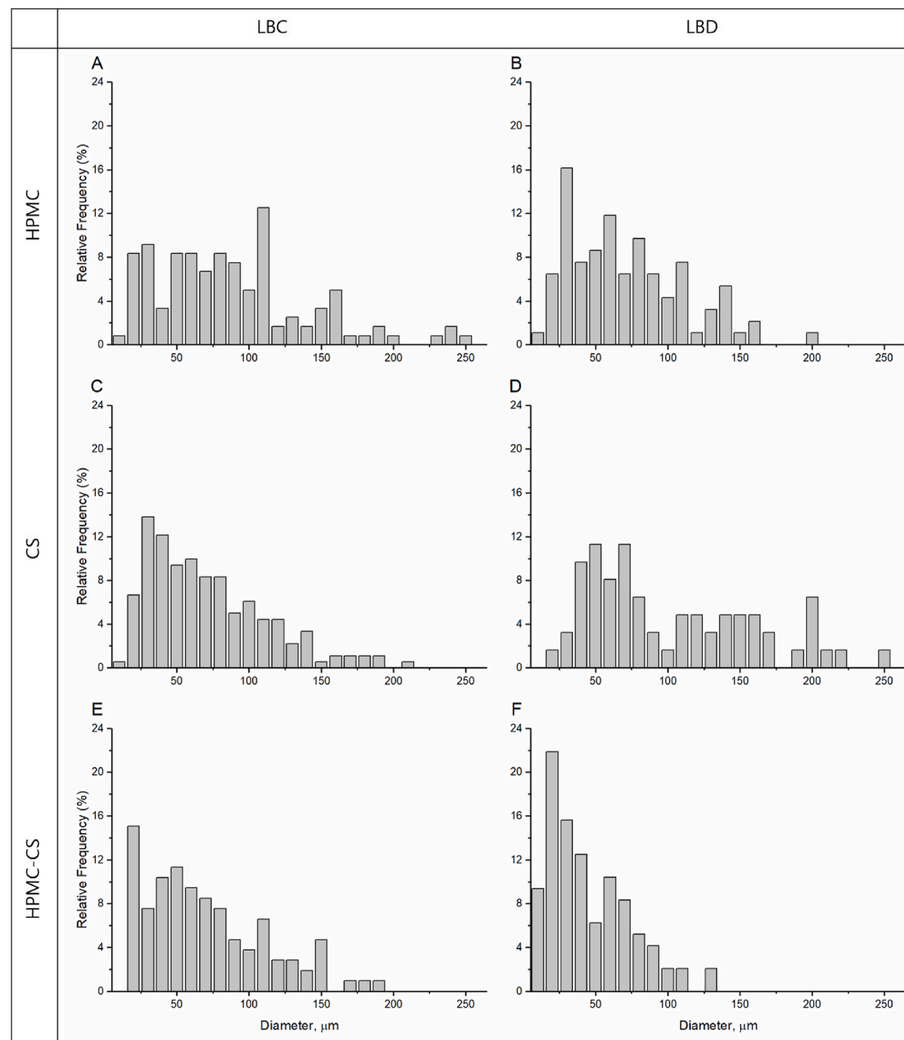


Fig. 6. Size distributions of air bubbles analyzed from CLSM micrographs of whipped cream created using HPMC (A, B), CS (C, D) and HPMC-CS (E, F) formulations containing LBC (A, C, E) and LBD (B, D, F) bacteria, respectively. Results were analyzed from at least 100 air bubbles.

microscopy that both LBC and LBD maintained their viability after whipping, based on their non-damaged cell membranes as indicated by green color. This discrepancy was further explained by observing the plating suspensions of LBC and LBD, where strong bacterial aggregation was observed for LBC but not for LBD (Fig. S4), thus demonstrating that one counted colony might represent an initial aggregate of bacteria rather than a single cell. Therefore, compared with non-aggregating LBD, the culturability of the LBC evaluated by CFU counting was somewhat underestimated, thereby indicating that neither the components nor the whipping process was found to cause detrimental effects on bacterial viability.

4. Discussion

In dairy whipping cream, fat globules sizing from 0.1 to 15 μm are dispersed in serum phase, forming oil-in-water emulsions that are kinetically and thermodynamically unstable (Fleming et al., 2017; Long et al., 2012). The small sizes and potential instability of fat globules contribute to their active structural roles during whipping process, by both adsorbing at air-water interfaces and building network as a result of partial coalescence. Likewise, lactic acid bacteria having similar sizes in the micron range and surface activity enabling their Pickering adsorption and self-aggregation in aqueous phase, can in principle be utilized as the alternative structural building blocks to fat globules. Regarding their Pickering capability, some strains of lactic acid bacteria were previously reported to adsorb at air-water interface and stabilize dry coarse foam with fast drainage and poor liquid retention ability (Falco et al., 2017; Jiang et al., 2019). In contrast to the reported dry foam, the whipped foam produced in the present work displayed more solid-like behavior and stiffness to support its own weight and ability to retain the serum phase in the foam structure. To the authors' knowledge, this is the first attempt to fully replace fat content with high load of edible lactic acid bacteria to formulate whipping cream with considerable foaming properties and water retention ability.

Almost all the samples showed higher air incorporation than the commercial dairy whipping cream. Interestingly, the two selected strains, despite exhibiting different surface properties in terms of cell hydrophobicity and aggregating properties, produced whipped cream with nearly identical overruns. Combined with the fact that air was not incorporated into the cell suspensions with only MQ water, the overrun was seemingly only attributed to the addition of two components, CS and HPMC, and the degree was based on the surface activities and their contribution to bulk viscosity (Alizadeh et al., 2019). With respect to the control samples without bacteria, it was not surprising that the CS solution displayed the highest overrun among the three formulations because of its lowest apparent viscosity (Fig. S1) and high surface activity (Sánchez et al., 2005). In comparison, the surface activity of HPMC molecules was reported to be even stronger than proteins such as

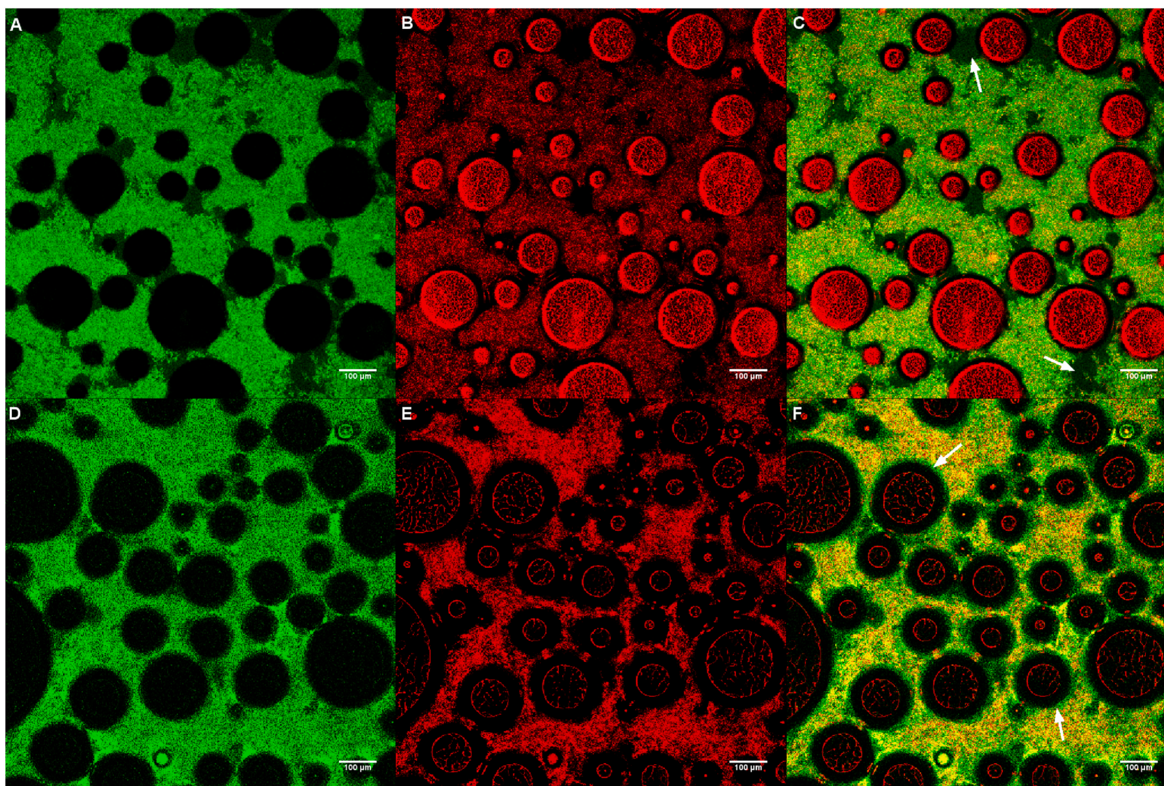


Fig. 7. CLSM micrographs showing the location of CS and bacteria in whipped cream of HPMC-CS formulation containing LBC (A, B, C) and LBD (D, E, F) bacteria, respectively. The CS component was stained using green-fluorescent FITC dye (A, D), while bacteria are highlighted in red using NucRed stain (B, E). The combined channels show that CS was almost only co-localized with LBC based on the weakly-fluorescent CS-alone areas (C), while in LBD samples, CS-alone phase of strong green fluorescence was observed surrounding the air bubbles (F). White arrows indicate the CS-alone areas. The scale bars represent 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

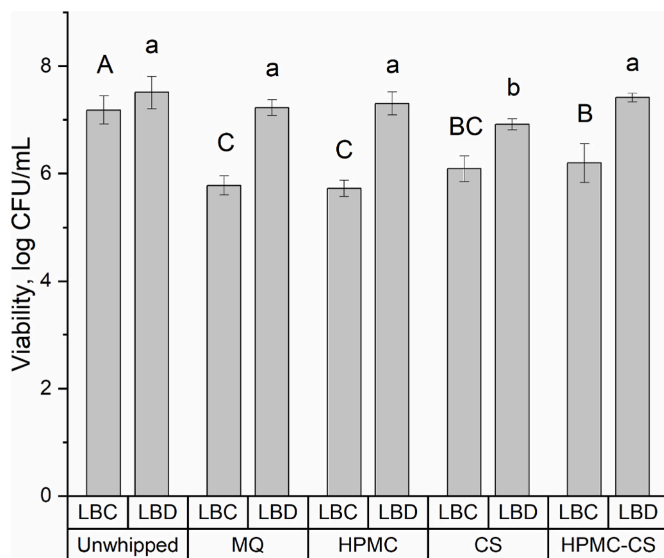


Fig. 8. Viability of bacteria in terms of CFU/mL count in unwhipped controls and whipped formulations with MQ water, HPMC, CS and HPMC-CS containing LBC and LBD bacteria, respectively. Error bars represent standard errors ($n = 2$). Lower case letters (a–c) indicate statistical grouping for LBD samples whereas upper case letters (A–C) are used for LBC samples. Different letters indicate significant differences ($P < 0.05$) from a given sample.

β-casein due to the presence of massive methyl groups on its backbone (Arboleya & Wilde, 2005; Pérez et al., 2008), and this complied well with our microscopic observation that the air bubbles were more wetted by the HPMC phase than the CS phase (Fig. 7C and F). On the other hand, the creation of overrun was not only related to the surface activity but also affected by the speed of molecular adsorption at air-water interface. Thus, the high bulk viscosity of HPMC seemed to dominate their slower adsorption, creating lower overrun of HPMC-containing controls compared to the CS control. The overrun affected by HPMC and CS components was better reflected in the CLSM graphs of LBD samples where bacterial Pickering effect was almost absent. In HPMC-containing samples, the dark areas of depletion flocculation by HPMC polymers were mostly located around the air bubbles, which in HPMC-CS sample was additionally adsorbed by a greenish CS layer, indicating the active role of both HPMC and CS in producing air bubbles for LBD samples. However, the highest overrun of CS formulations was not captured for the LBD sample due to the fast bubble coalescence occurring from the low bulk viscosity and fast drainage. However, for LBC samples, the strong Pickering effect of LBC compensated for the drainage instability of the CS formulation, and therefore air bubbles with a small and narrow size distribution was still observed. Otherwise, no evident size difference in air bubbles was found between LBC and LBD samples, indicating again that the initial air incorporation was hardly affected by the surface properties and Pickering capacity of involved strains.

Unlike the results of the overrun, the drainage stability was greatly dependent on the surface properties of bacteria, although the bulk viscosity also played a role, as seen from the most severe drainage of CS formulation, complying with their fast phase separation and destabilization observed in the overrun measurement. Liquid drainage was differently prevented by adding LBC and LBD bacteria. The drainage stability was most likely maintained by two contributory factors which included the local viscosity and capillary effects. An overall higher stability of LBC samples may be explained by the strong Pickering effect of

LBC, where their irreversible adsorption increased the local shear rheology of the air-water interface that was initially formed by CS and HPMC components; this eventually imparted foam stability by inhibiting drainage and rupture of liquid film (Dickinson, 2017; Murray et al., 2011). Likewise, LBD samples containing HPMC exhibited much higher drainage stability than the CS-only sample also due to the higher bulk viscosity and local viscosity of liquid film ensured by HPMC polymer. Besides the dominance of viscosity, capillary effects induced by the aggregation of non-adsorbed bacteria might also play roles in holding liquid inside the foam structure. Indeed, compared with LBD, LBC exhibited apparently stronger aggregation in the whipped foam, independent on formulations, and therefore drainage was markedly inhibited in LBC samples due to the entanglements of irregular bacterial aggregates in continuous phase, eventually better holding liquid inside the solid network through capillary effects. Even though the amount of non-adsorbed LBC cells was diminished due to their high adsorption at air-water interfaces, the strong aggregation ability of hydrophobic LBC still mimicked the partial coalescence of fat globules in continuous phase.

Besides drainage stability, the whipped cream also needs to display a solid-like behavior to stand and support its own weight, which is in turn determined by the strength of solid network, in this case, the aggregation of bacteria and subsequent network formation. Although bacterial aggregation is a complex outcome of multiple factors, it could be still understood from a physicochemical point of view through the surface properties of the bacteria. Both the aggregation assay and static light scattering of fresh whipping cream demonstrated the comparable aggregation of LBC and LBD in the initial time, whereas LBC tended to show stronger aggregation along with storage time and whipping process. Considering the similar electrostatic effects shown by two strains, the difference in their long-term aggregation was most likely driven by the steric forces and hydrophobic interactions. To elaborate, the high surface hydrophobicity of LBC was primarily rendered by the presence of surface-layer proteins, which are regular crystalline arrays covering the cell envelope (Smit et al., 2001; Toba et al., 1995). Therefore, compared with LBD lacking such structures (Greene & Klaenhammer, 1994), the steric interactions of LBC was much weaker due to high surface compactness, which combined with their stronger hydrophobic attractions between cells, ultimately led to more severe aggregation of LBC than LBD. After whipping, the considerably higher G′ values of LBC samples than LBD samples confirmed the higher strength of LBC aggregates resulting in more solid-like properties, whereas in the meantime a higher damping of LBC samples simultaneously indicated somewhat liquid-like characters, probably due to their stronger ability to also retain liquid inside. Furthermore, the two added components, CS and HPMC, displayed distinctly different functions in systems of LBC and LBD. Based on the CLSM results, the CS component seemed to show affinity to both strains according to their co-localization with bacteria potentially via hydrophobic interactions (Ly et al., 2008), which explained the stronger interactions of CS with the hydrophobic LBC than the hydrophilic LBD. This further contributed to the strong bacterial aggregation in LBC samples, and therefore CS-containing LBC samples typically resulted in larger bacterial aggregates and higher G′ values. In contrast, HPMC played a more predominant role in creating stiffness of LBD samples, as seen from the higher G’ values of HPMC-containing formulations, potentially by inducing depletion flocculation of bacteria. This effect was clearly reflected in CLSM graphs of LBD samples that bacteria were squeezed away near the HPMC-rich areas of non-fluorescent dark rings, whereas the depletion flocculation was less pronounced for LBC samples according to the nearly unaffected dark areas by HPMC, also possibly indicating a higher compatibility of LBC and HPMC in the continuous phase.

In the present study, the two representative strains LBC and LBD, showing similar zeta potential but different hydrophobicity, were selected from the local strain collection, and they can be in principle replaced by any other strains or even species showing appropriate surface properties such as Pickering adsorption and cell aggregation. Together with the bacteria, proper hydrocolloids should be combined to further promote the air incorporation, bulk viscosity and bacterial aggregation. The areas depleted of bacteria should mostly appear in the continuous phase instead of on the air-water interface, and thus allowing direct contact between aggregated bacteria and air-bubbles and this would better contribute to the building of bacterial network mimicking the structural role of solid fat. Moreover, the demonstration of food formulations based on such large quantity of lactic acid bacteria leads to the questions of the safety and nutritional aspects of lactic acid bacteria. First, lactic acid bacteria are Generally Recognized as Safe (GRAS) (Åvall-Jääskeläinen & Palva, 2005) and their use in foods can also bring additional functionalities such as the enhancement of flavor, and potential health-promoting effects by inhibiting the colonization and toxin production of pathogenic or spoilage bacteria in human gastrointestinal tract (Wallis et al., 2019). Even though previous studies have assigned the energy contents of many bacteria and yeasts (Prochazka et al., 1970, 1973), the values will only be consumed if the bacteria are fully digested rather than colonizing in human intestine and excreted afterwards. Therefore, it is believed that using lactic acid bacteria as structural building blocks might lower the food energy density compared with colloidal foods based on fat globules. However, this should be investigated separately in future. Further, the economic feasibility of the concept can also be a consideration, as lactic acid bacteria are normally used in small quantity in foods as either starting cultures or probiotic ingredients, and their production involves highly-refined carbohydrates which makes them rather high-value products. In order to be used as structural building blocks in large quantities, more focuses should be put on the large-scale production of lactic acid bacteria based on less-refined raw materials from e.g. side stream, and thus obtaining a better cost efficiency. On top of that, the unchanged viability in the present study and reported probiotic properties of LBC and LBD (Li et al., 2021; Moro-garcía et al., 2013) can provide additional insight into the development of functional colloidal foods with enhanced health-promoting effects. Future studies could include monitoring bacterial viability over a longer and more realistic storage period prior to whipping and consumption of the product.

5. Conclusion

Non-fat whipping cream analogues with high overrun and drainage stability were formulated using lactic acid bacteria as natural structural building blocks with the combination of CS and HPMC components. Overall, the major achievement of this work was not the creation of foam that can be done by using any surface-active component, but the formation of wet, slow-draining and stiff foam with the standing ability to support its own weight, and such functionality traditionally achieved by saturated fats was successfully obtained by using lactic acid bacteria with aggregating ability. More hydrophobic LBC strain showing stronger aggregating properties, created whipped cream with higher stiffness and liquid retention ability than the hydrophilic LBD strain, due to the strong Pickering stabilizing capacity and network forming capability of LBC bacteria. The aggregation of two strains was differently enhanced by adding components HPMC and CS in the formulated system, where CS better promoted the aggregation of LBC via strong hydrophobic interactions, while HPMC induced stronger aggregation of LBD due to the effect of depletion flocculation. This yielded higher stiffness of the corresponding formulations containing LBC and LBD. However, the initial air incorporation of whipped cream was seen independent on the involved strain, which was instead only determined by the properties of HPMC and CS regarding their surface activities and improvements in bulk viscosity. Considering that neither the added components nor the whipping process caused detrimental effects on bacterial viability, together with the weakly-acidic pH and nitrogen source present in the formulated systems, potential strategies can be developed in the future to ensure the survival and even proliferation of bacteria in the whipped

structures after processing or long-term storage. This will further open the possibility of using the natural lactic acid bacteria as both structural building blocks and probiotic food components.

CRediT authorship contribution statement

Xiaoyi Jiang: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft. **Elhamalsadat Shekarforoush:** Methodology, Writing – review & editing. **Musemma Kedir Muhammed:** Methodology, Writing – review & editing. **Kathryn A. Whitehead:** Writing – review & editing. **Nils Arneborg:** Writing – review & editing, Supervision. **Jens Risbo:** Conceptualization, Writing – review & editing, Supervision, project management, Funding acquisition.

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.

Acknowledgements

The research leading to these results has received funding from The Danish Independent Research Foundation under framework grant n° 8022-00139B and financial support of Chinese Scholarship Council (CSC) grant n° 201807940009.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2022.108137.

References

Alizadeh, L., Abdolmaleki, K., Nayebzadeh, K., & Bahmaei, M. (2019). Characterization of sodium caseinate/Hydroxypropyl methylcellulose concentrated emulsions : Effect of mixing ratio , concentration and wax addition. *International Journal of Biological Macromolecules, 128*, 796–803. https://doi.org/10.1016/j.ijbiomac.2019.01.208

Allen, K. E., Murray, B. S., & Dickinson, E. (2008). Whipped cream-like textured systems based on acidified caseinate-stabilized oil-in-water emulsions. *International Dairy Journal, 18*(10–11), 1011–1021. https://doi.org/10.1016/j.idairyj.2008.04.003

Anna, M., Bibat, D., Jasmin, M., & Eun, J. (2022). Impact of replacing pork backfat with rapeseed oleosomes – natural pre-emulsified oil – on technological properties of meat model systems. *Meat Science, 186*(January), Article 108732. https://doi.org/10.1016/j.meatsci.2021.108732

Arboleya, J. C., & Wilde, P. J. (2005). Competitive adsorption of proteins with methylcellulose and hydroxypropyl methylcellulose. *Food Hydrocolloids, 19*(3), 485–491. https://doi.org/10.1016/j.foodhyd.2004.10.013

Athari, B., Nasirpour, A., Saeidy, S., & Esehaghbeygi, A. (2021). Physicochemical properties of whipped cream stabilized with electrohydrodynamic modified cellulose. *Journal of Food Processing and Preservation, 45*(9), 1–8. https://doi.org/10.1111/jfpp.15688

Åvall-Jääskeläinen, S., & Palva, A. (2005). Lactobacillus surface layers and their applications. *FEMS Microbiology Reviews, 29*(3 SPEC. ISS.), 511–529. https://doi.org/10.1016/j.femsre.2005.04.003

Bancalari, E., Gatti, M., Bottari, B., Mora, D., & Arioli, S. (2022). Disclosing Lactobacillus delbrueckii subsp. bulgaricus intraspecific diversity in exopolysaccharides production. *Food Microbiology, 102*(June 2021), Article 103924. https://doi.org/10.1016/j.fm.2021.103924

Bellon-Fontaine, M. N., Rault, J., & Van Oss, C. J. (1996). Microbial adhesion to solvents: A novel method to determine the electron-donor/electron-acceptor or Lewis acid-base properties of microbial cells. *Colloids and Surfaces B: Biointerfaces, 7*, 47–53. https://doi.org/10.1016/0927-7765(96)01272-6

Binks, B. P. (2002). Particles as surfactants -similarities and differences. *Current Opinion in Colloid & Interface Science, 7*, 21–41. https://doi.org/10.2118/141256-PA

Bruhn, C. M., & Bruhn, J. C. (1988). Observations on the whipping characteristics of cream. *Journal of Dairy Science, 71*(3), 857–862. https://doi.org/10.3168/jds.S0022-0302(88)79628-9

Dickinson, E. (2010). Food emulsions and foams: Stabilization by particles. *Current Opinion in Colloid & Interface Science, 15*(1–2), 40–49. https://doi.org/10.1016/j.cocis.2009.11.001

Dickinson, E. (2012). Use of nanoparticles and microparticles in the formation and stabilization of food emulsions. *Trends in Food Science & Technology, 24*(1), 4–12. https://doi.org/10.1016/j.tifs.2011.09.006

Dickinson, E. (2017). Biopolymer-based particles as stabilizing agents for emulsions and foams. *Food Hydrocolloids, 68*, 219–231. https://doi.org/10.1016/j.foodhyd.2016.06.024

Falco, C. Y., Geng, X., Cárdenas, M., & Risbo, J. (2017). Edible foam based on Pickering effect of probiotic bacteria and milk proteins. *Food Hydrocolloids, 70*, 211–218. https://doi.org/10.1016/j.foodhyd.2017.04.003

Feichtinger, A., & Scholten, E. (2020). Preparation of protein oleogels: Effect on structure and functionality. *Foods, 9*(12), 1745. https://doi.org/10.3390/foods9121745

Firoozmand, H., & Rousseau, D. (2016). Microbial cells as colloidal particles : Pickering oil-in-water emulsions stabilized by bacteria and yeast. *Food Research International, 81*, 66–73. https://doi.org/10.1016/j.foodres.2015.10.018

Fleming, A., Schenkel, F. S., Chen, J., Malchiodi, F., Ali, R. A., Mallard, B., Sargolzaei, M., Corredig, M., & Miglior, F. (2017). Variation in fat globule size in bovine milk and its prediction using mid-infrared spectroscopy. *Journal of Dairy Science, 100*(3), 1640–1649. https://doi.org/10.3168/jds.2016-11427

Ghosh, S., & Rousseau, D. (2011). Fat crystals and water-in-oil emulsion stability. *Current Opinion in Colloid & Interface Science, 16*(5), 421–431. https://doi.org/10.1016/j.cocis.2011.06.006

Ghribi, A. M., Zouari, M., Attia, H., & Besbes, S. (2021). Study of protein/k-carrageenan mixture's effect on low-fat whipping cream formulation. *Lebensmittel-Wissenschaft & Technologie, 147*(May), Article 111647. https://doi.org/10.1016/j.lwt.2021.111647

Greene, J. D., & Klaenhammer, T. R. (1994). Factors involved in adherence of lactobacilli to human Caco-2 cells. *Applied and Enviromental Microbiology, 60*(12), 4487–4494.

Gupta, R., & Rousseau, D. (2012). Surface-active solid lipid nanoparticles as Pickering stabilizers for oil-in-water emulsions. *Food & Function*, 302–311. https://doi.org/10.1039/c2fo10203j

Hunter, T. N., Pugh, R. J., Franks, G. V., & Jameson, G. J. (2008). The role of particles in stabilising foams and emulsions. *Advances in Colloid and Interface Science, 137*(2), 57–81. https://doi.org/10.1016/j.cis.2007.07.007

Iftikhar, S. A., & Dutta, H. (2020). Use of raw and physically modified rice starches as fat replacer in whipping cream. *Current Research in Nutrition and Food Science, 8*(1), 122–130. https://doi.org/10.12944/CRNFSJ.8.1.11

Jiang, X., Falco, C. Y., Dalby, K. N., Siegumfeldt, H., Arneborg, N., & Risbo, J. (2019). Surface engineered bacteria as Pickering stabilizers for foams and emulsions. *Food Hydrocolloids, 89*(October 2018), 224–233. https://doi.org/10.1016/j.foodhyd.2018.10.044

Jiang, X., Shekarforoush, E., Muhammed, M. K., Whitehead, K., Simonsen, A. C., Arneborg, N., & Risbo, J. (2021). Efficient chemical hydrophobization of lactic acid bacteria – one-step formation of double emulsion. *Food Research International, 147* (February), Article 110460. https://doi.org/10.1016/j.foodres.2021.110460

Jonsson, P., & Wadström, T. (1984). Cell surface hydrophobicity of Staphylococcus aureus measured by the salt aggregation test (SAT). *Current Microbiology, 10*(4), 203–209. https://doi.org/10.1007/BF01627256

Leone, L., Loring, J., Sjöberg, S., Persson, P., & Shchukarev, A. (2006). Surface characterization of the Gram-positive bacteria Bacillus subtilis-an XPS study. *Surface and Interface Analysis, 38*(4), 202–205. https://doi.org/10.1002/sia.2184

Li, P., Wei, K., He, X., Zhang, L., Liu, Z., Wei, J., & Chen, X. (2021). Vaginal probiotic Lactobacillus crispatus seems to inhibit sperm activity and subsequently reduces pregnancies in rat. https://doi.org/10.3389/fcell.2021.705690, 1-14.

Long, Z., Zhao, M., Zhao, Q., Yang, B., & Liu, L. (2012). Effect of homogenisation and storage time on surface and rheology properties of whipping cream. *Food Chemistry, 131*(3), 748–753. https://doi.org/10.1016/j.foodchem.2011.09.028

van Loosdrecht, M. C., Lyklema, J., Norde, W., Schraa, G., & Zehnder, A. J. (1987). The role of bacterial cell wall hydrophobicity in adhesion. *Applied and Environmental Microbiology, 53*(8), 1893–1897. https://doi.org/10.1128/aem.53.8.1893-1897.1987

Ly, M. H., Aguedo, M., Goudot, S., Le, M. L., Cayot, P., Teixeira, J. A., Le, T. M., Belin, J. M., & Waché, Y. (2008). Interactions between bacterial surfaces and milk proteins, impact on food emulsions stability. *Food Hydrocolloids, 22*(5), 742–751. https://doi.org/10.1016/j.foodhyd.2007.03.001

Marín, M. L., Benito, Y., Pin, C., Fernández, M. F., García, M. L., Selgas, M. D., & Casas, C. (1997). Lactic acid bacteria: Hydrophobicity and strength of attachment to meat surfaces. *Letters in Applied Microbiology, 24*(1), 14–18. https://doi.org/10.1046/j.1472-765X.1997.00334.x

Marinova, K. G., Basheva, E. S., Nenova, B., Temelska, M., Mirarefi, A. Y., Campbell, B., & Ivanov, I. B. (2009). Physico-chemical factors controlling the foamability and foam stability of milk proteins : Sodium caseinate and whey protein concentrates. *Food Hydrocolloids, 23*(7), 1864–1876. https://doi.org/10.1016/j.foodhyd.2009.03.003

Mert, B., & Vilgis, T. A. (2021). Food Hydrocolloids Hydrocolloid coated oleosomes for development of oleogels. *Food Hydrocolloids, 119*(February), Article 106832. https://doi.org/10.1016/j.foodhyd.2021.106832

Millsap, K., Reid, G., van der Mei, H. C., & Busscher, H. J. (1997). Cluster analysis of genotypically characterized Lactobacillus species based on physicochemical cell surface properties and their relationship with adhesion to hexadecane. *Canadian Journal of Microbiology, 43*, 284–291. https://doi.org/10.1139/m97-039

Mobili, P., de los Ángeles Serradell, M., Trejo, S. A., Avilés Puigvert, F. X., Abraham, A. G., & De Antoni, G. L. (2009). Heterogeneity of S-layer proteins from aggregating and non-aggregating Lactobacillus kefir strains. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology, 95*(4), 363–372. https://doi.org/10.1007/s10482-009-9322-y

Moro-garcía, M. A., Alonso-arias, R., Baltadjieva, M., Benítez, C. F., Amadeo, M., Barrial, F., Ruisánchez, E. D., Santos, R. A., Sánchez, M.Á., Miján, J. S., & López-larrea, C. (2013). Oral supplementation with Lactobacillus delbrueckii subsp . bulgaricus 8481 enhances systemic immunity in elderly subjects. https://doi.org/10.1007/s11357-012-9434-6, 1311-1326.

Murray, B. S., Durga, K., Yusoff, A., & Stoyanov, S. D. (2011). Stabilization of foams and emulsions by mixtures of surface active food-grade particles and proteins. *Food Hydrocolloids, 25*(4), 627–638. https://doi.org/10.1016/j.foodhyd.2010.07.025

Nikiforidis, C. V. (2019). Structure and functions of oleosomes (oil bodies). *Advances in Colloid and Interface Science, 274*, Article 102039. https://doi.org/10.1016/j.cis.2019.102039

Ojeda, J. J., Romero-González, M. E., Bachmann, R. T., Edyvean, R. G. J., & Banwart, S. A. (2008). Characterization of the cell surface and cell wall chemistry of drinking water bacteria by combining XPS, FTIR spectroscopy, modeling, and potentiometric titrations. *Langmuir, 24*(8), 4032–4040. https://doi.org/10.1021/la702284b

Peng, F., He, S., Yi, H., Li, Q., Xu, W., Wang, R., & Ma, Y. (2018). Physical, textural, and rheological properties of whipped cream affected by milk fat globule membrane protein. *International Journal of Food Properties, 21*(1), 1190–1202. https://doi.org/10.1080/10942912.2018.1460755

Pérez, O. E., Sánchez, C. C., Pilosof, A. M. R., & Rodríguez Patino, J. M. (2008). Dynamics of adsorption of hydroxypropyl methylcellulose at the air-water interface. *Food Hydrocolloids, 22*(3), 387–402. https://doi.org/10.1016/j.foodhyd.2006.12.005

Polak-Berecka, M., Wasko, A., Paduch, R., Skrzypek, T., & Sroka-bartnicka, A. (2014). The effect of cell surface components on adhesion ability of Lactobacillus rhamnosus. *Antonie Van Leeuwenhoek*, 751–762. https://doi.org/10.1007/s10482-014-0245-x

Prochazka, G. J., Payne, W. J., & Mayberry, W. R. (1970). Calorific content of certain bacteria and fungi. *Journal of Bacteriology, 104*(2), 646–649.

Prochazka, G. J., Payne, W. J., & Mayberry, W. R. (1973). Calorific contents of microorganisms. *Biotechnology and Bioengineering, 15*, 1007–1010.

Rayner, M., Marku, D., Eriksson, M., Sjöö, M., Dejmek, P., & Wahlgren, M. (2014). Biomass-based particles for the formulation of Pickering type emulsions in food and topical applications. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 458*, 48–62. https://doi.org/10.1016/j.colsurfa.2014.03.053

Reid, G., Bialkowska-Hobrzanska, H., Van Der Mei, H. C., & Busscher, H. J. (1999). Correlation between genetic, physico-chemical surface characteristics and adhesion of four strains of Lactobacillus. *Colloids and Surfaces B: Biointerfaces, 13*(2), 75–81. https://doi.org/10.1016/S0927-7765(98)00107-6

Rezvani, F., Abbasi, H., & Nourani, M. (2020). Effects of protein–polysaccharide interactions on the physical and textural characteristics of low-fat whipped cream. *Journal of Food Processing and Preservation, 44*(10), 1–12. https://doi.org/10.1111/jfpp.14743

Romero-guzmán, M. J., Köllmann, N., Zhang, L., Boom, R. M., & Nikiforidis, C. V. (2020). Controlled oleosome extraction to produce a plant-based mayonnaise-like emulsion using solely rapeseed seeds. *LWT - Food Science and Technology, 123*, Article 109120. https://doi.org/10.1016/j.lwt.2020.109120. August 2019),.

Salahi, M. R., & Mohebbi, M. (2021). Development of soy milk in the form of wet foam in the presences of whey protein concentrate and polysaccharides at different whipping temperatures: Study of physical, rheological and microstructural properties. *Lebensmittel-Wissenschaft & Technologie, 137*. https://doi.org/10.1016/j.lwt.2020.110444

Sánchez, C. C., Rodríguez Niño, R., Caro, A. L., & Rodríguez Patino, J. M. (2005). Biopolymers and emulsifiers at the air-water interface. Implications in food colloid formulations. *Journal of Food Engineering, 67*, 225–234. https://doi.org/10.1016/j.jfoodeng.2004.05.065

Shalaby, S., Farahat, A., & Heikal, Y. (2013). Rheological and physical properties of low-fat whipped cream containing dried lupine powder and whey protein concentrate. *Egyptian Journal of Dairy Science, 41*, 181–191.

Smit, E., Oling, F., Demel, R., Martinez, B., & Pouwels, P. H. (2001). The S-layer protein of Lactobacillus acidophilus ATCC 4356: Identification and characterisation of domains responsible for S-protein assembly and cell wall binding. *Journal of Molecular Biology, 305*(2), 245–257. https://doi.org/10.1006/jmbi.2000.4258

Toba, T., Virkola, R., Westerlund, B., Bjorkman, Y., Sillanpaa, J., Vartio, T., Kalkkinen, N., & Korhonen, T. K. (1995). A collagen-binding S-layer protein in Lactobacillus crispatus. *Applied and Environmental Microbiology, 61*(7), 2467–2471. https://doi.org/10.1128/aem.61.7.2467-2471.1995

Vadillo-Rodríguez, V., Busscher, H. J., Norde, W., De Vries, J., & Van Der Mei, H. C. (2004). Dynamic cell surface hydrophobicity of Lactobacillus strains with and without surface layer proteins. *Journal of Bacteriology, 186*(19), 6647–6650. https://doi.org/10.1128/JB.186.19.6647-6650.2004

Wallis, J. K., Krömker, V., & Paduch, J. H. (2019). Biofilm challenge: Lactic acid bacteria isolated from bovine udders versus staphylococci. *Foods, 8*(2). https://doi.org/10.3390/foods8020079

Wang, C., He, X. W., Huang, Q., Fu, X., & Liu, S. (2013). Physicochemical properties and application of micronized cornstarch in low fat cream. *Journal of Food Engineering, 116*(4), 881–888. https://doi.org/10.1016/j.jfoodeng.2013.01.025