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Oleogel-Structured Emulsion With Protein-Polysaccharide Complex: Impact on Stability of Cacao Whey-Based Beverage

*[1]Rasha, A. Ibrahim, [1]Abeer, F. Zayan, [1]Tamer, S. Abdulalim & [2]Amr, M. Bakry

[1]Dairy Technology Research Department, Food Technology Research Institute, Agricultural Research Centre, Giza 12619, Egypt

[2] Dairy Science Department, Faculty of Agriculture, New Valley University, New Valley, El-Kharga 72511, Egypt

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ABSTRACT

The development of functional dairy beverages (FDBs) faces significant challenges related to structural stability and nutritional enhancement. Innovative approaches, such as the use of oleogels and protein-polysaccharide complexes, offer viable alternatives for improving the texture, stability, and nutritional profile of these products. This research examines the effects of oleogel-structured emulsions and protein-polysaccharide complexes on the stability and quality attributes of cacao-based whey beverages (CWBs). The findings indicate that the incorporation of oleogel and okra mucilage significantly influenced the rheological behavior and microstructure of the CWB formulations. Samples with higher mucilage content (0.3% w/w) exhibited smaller particle sizes, narrower size distributions, and improved emulsion stability compared to formulations with lower mucilage content (0.2% w/w). Transmission electron microscopy (TEM) analysis revealed the formation of a distinct core-shell structure, where the oleogel core was surrounded by a protein-polysaccharide interfacial layer, contributing to enhanced physical stability. Furthermore, the modified CWB formulations demonstrated a favorable fatty acid profile, characterized by increased unsaturated fatty acids and reduced levels of saturated and trans fatty acids. This led to improved nutritional indices, such as the atherogenic index (AI) and thrombogenic index (TI), indicating potential health benefits. No significant differences in total bacterial counts were observed among the beverage treatments throughout the 14-day storage period at 5°C. These results suggest that the incorporation of oleogel-structured emulsions and protein-polysaccharide complexes can effectively enhance the stability, texture, and nutritional quality of cacao-based whey beverages. The findings provide valuable insights for the development of innovative and functional dairy products.

1. Introduction

Whey is a common byproduct of the dairy industry, particularly generated during cheese production through the coagulation of casein (Bandara et al., 2023). It holds significant value, as more than 88% of the milk volume used in cheese making is converted into whey. Remarkably, over half of the nutrients originally present in milk are retained in whey (Uald Lamkaddam et al., 2023). Globally, approximately twenty-one million tons of whey are produced annually from the manufacture of twenty-four million tons of cheese (Lopes et al., 2019). More than half of the whey generated worldwide is utilized in various products, while the remainder is either discarded as waste or used as animal feed (Bandara et al., 2023). Beverage production has emerged as a promising and efficient approach to whey valorization. Moreover, current trends clearly growing emphasis on promoting consumer health and well-being (Ribeiro et al., 2021). In addition to fortified inriched beverages containing novel bioactive components, functional beverages whether derived from plant or animal sources are recognized for their therapeuticpotential, which goes beyond

*Corresponding Author
Email: rashamorsy@arc.sci.eg

basic nutritional needs (Mehra et al., 2021). Plant-derived oils and fats account for at least 79% of the total annual global output (Bordón et al., 2019). Oils rich in omega-3 (ω-3) fatty acids, when included in the diet, are highly effective in preventing and managing various health conditions such as coronary heart disease, diabetes, and cancer (Fujii et al., 2021). Flaxseed oil, for example, contains 88.97% unsaturated fatty acids and is particularly abundant in omega-3 fatty acids—mainly alpha-linolenic acid (ALA). This supports infant brain development, improves blood lipid profiles, prevents cardiovascular diseases, and provides anti-inflammatory benefits (Bordón et al., 2019 and Lu et al., 2020). However, it contains 11.01% saturated fatty acids and is prone to oxidation, especially at room temperature (Edel et al., 2015). Extra virgin olive oil is considered a functional nutraceutical due to its bioactive compounds (Gambino et al., 2018). It is composed primarily of palmitic, stearic, oleic, and linoleic fatty acids (Uceda et al., 2008), and contains 1–2% phenolic compounds, phytosterols, tocopherols, and squalene. Additionally, it includes other bioactive micro-components with health benefits, such as vitamin E and cardioprotective compounds like hydroxytyrosol, oleuropein, oleocanthal, and lignans (Martinéz et al., 2006; Oliveira et al., 2024). Emulsion formulations derived from plant-based oils such as flaxseed, soybean, coconut, and olive oil may contribute to the prevention of inflammation, cardiovascular diseases, and metabolic syndrome. This is attributed to the presence of polyunsaturated fatty acids and various bioactive compounds with antioxidant, anti-inflammatory, and immunomodulatory properties (Santangelo et al., 2018; Pradelli et al., 2023; Tsamesidis & Kalogianni, 2023; Oliveira et al., 2024). Oil-in-water emulsions are more easily incorporated into water-based food products including beverages, dairy items, salad dressings, baked goods, and protein-rich foods compared to bulk oils, which tend to separate during storage. These emulsions are thermodynamically unstable and subject to degradation due to gravitational separation, flocculation, coalescence, and Ostwald ripening (McClements, 2004). However, their kinetic stability can be enhanced using encapsulating agents and emulsifiers. Emulsifiers are surface-active molecules that adsorb to oil droplet surfaces during homogenization, creating a barrier that prevents droplet aggregation. Proteins are commonly used in food emulsions due to their amphiphilic properties, enabling them to provide both steric and electrostatic repulsion between oil droplets, as well as forming a strong interfacial membrane to prevent flocculation and coalescence during storage (McClements, 2004). Among these, whey proteins are particularly favored for their hydrophobic and hydrophilic balance (Goyal et al., 2015).Another class of promising delivery and texturizing materials in food systems is oleogels. Oleogels are oil-based gels formed using structuring agents such as beeswax, which trap healthy liquid oils within a thermo-reversible three-dimensional matrix. These gels not only enhance texture and prevent sedimentation, but also reduce the content of saturated and trans fats, while enabling the delivery of bioactive compounds (Ögütcü & Yilmaz, 2015; Meissner et al., 2020; Park et al., 2022; Huang et al., 2023; Wang et al., 2024). Although research on the interaction of oleogels with proteins is still emerging, protein-based oleogels also known as proteinogen oleogels represent a novel category of organogels. These are characterized by a three-dimensional network that traps water and organizes liquid oil into a semi-solid system with viscoelastic, gel-like properties (Meissner et al., 2020; Plazzotta et al., 2023). Polysaccharides are natural, high-molecular-weight polymers composed of monosaccharide units linked by glycosidic bonds. They are water-soluble and commonly used as thickeners, gelling agents, emulsifiers, foam stabilizers, and delivery systems for bioactive compounds (Bakry et al., 2016; Tudu & Samanta, 2023). Some polysaccharides also function as surfactants or emulsifiers, particularly in encapsulating active ingredients (Bakry et al., 2016; Wijekoon et al., 2023; Raj et al., 2024). Okra mucilage, a polysaccharide of natural origin, is non-toxic, biodegradable, and biocompatible. Its anionic nature and ability to form a thick adsorbed layer make it an excellent thickener and emulsifier for stabilizing oil-in-water emulsions (Raj et al., 2020; Yue et al., 2024). Recent studies have highlighted the synergistic behavior of polysaccharide–protein complexes in

stabilizing colloidal systems. These complexes contribute to interfacial stabilization and bulk structuring by forming mesoscopic building blocks such as colloidal particles, emulsion droplets, and gel networks (Wijaya et al., 2017). Despite these advances, the availability of innovative dairy-based beverages remains limited due to the restricted number of approved food emulsifiers capable of providing both thermodynamic and kinetic stability while meeting industrial standards for functionality and quality (e.g., consistency and appearance). The study aimed to develop a functional whey-based dairy beverage containing oil-in-water emulsions by leveraging the synergistic interactions among whey protein, okra-derived polysaccharides, and oleogels. The formulation process involved optimizing the ratios of protein, polysaccharide, and oleogel to achieve the desired physicochemical stability, sensory quality, and nutritional functionality. The stability of the emulsions was further evaluated under different oil types, preparation methods, and concentrations.

2. Materials and Methods

Materials

The source of fresh acid whey (AW) used in the production of cacao whey beverages was the Dairy Unit of the Faculty of Agriculture, Cairo University, Egypt. This whey was obtained as a by-product from the production of Karish cheese. After collection, the whey was stored at −18°C until further processing. The composition of the fresh acid whey was as follows: 6.42% total solids, 4.40% lactose, 0.53% protein, 0.98% ash, and 0.05% fat, with a pH of 4.30. The emulsifier mixture used in the formulation included soy lecithin (Adlec for Food Use, E322), acquired from ADM, Hamburg, Germany. Food-grade mono- and diglycerides (Grindsted® Mono-Di HV 52 K-A) were obtained from DuPont, NHIB, ApS Langebrogade 1, Denmark. Whey protein isolate (WPI) was sourced from Davisco Foods International, Inc., Minnesota, USA. Cacao powder was purchased from Cadbury-Mondelez Egypt Food Company, Cairo, Egypt. Sugarcane was donated by the governmental sugar company located in El-Howamdia, Giza, Egypt. Palm shortening, with a melting point of 45°C, was obtained from Arma Company, Cairo, Egypt. Flaxseed oil and olive oil were purchased from Sekem Group Company, Cairo, Egypt. Fresh okra was acquired from PICO Modern Agriculture Company, Giza, Egypt. Beeswax (a pale solid in pellet form with a subtle odor and a melting point of 62–65°C; free from arsenic, lead, and mercury; and classified as a GRAS additive) was sourced from the Bee Keeping Research Center, Faculty of Agriculture, Cairo University. Additional chemicals including sodium metabisulphite, ethyl alcohol (99.5%), NaOH, and phosphotungstic acid were obtained from Sigma-Aldrich, Gillingham, Dorset, UK.

Methods

Physicochemical analysis of acid whey and cacao whey beverages

The total solids, fat content (using the Soxhlet method), total carbohydrates, protein content (using the micro-Kjeldahl method), ash, and pH were analyzed according to the methodologies outlined by the Association of Official Analytical Chemists (AOAC, 2012). The lactose content in whey was determined following the method described by Lawrence (1968).

Extraction process of mucilage from okra

Before the extraction process, okra pods were thoroughly washed to remove any external dirt. The cleaned pods, with seeds removed, were promptly frozen and stored at −20°C to prevent color changes due to natural oxidation and browning. The extraction of okra mucilage was carried out based on the method described by Ameena et al. (2010), with minor modifications. One kilogram of cleaned and sliced okra at various maturity stages was mixed with distilled water at 5 °C containing 1% (w/v) sodium metabisulphite. The mixture (slurry) was then centrifuged at 3000 rpm for 5 minutes. Mucilage was precipitated from the supernatant using 99.5% ethyl alcohol. The precipitated mucilage was washed several times with acetone and air-dried in a drying oven (Venticell, MMM Medcenter Einrichtungen GmbH, München, Germany). The dried mucilage was then ground into powder using a commercial dry blender. Figure 1 illustrates the steps involved in extracting okra mucilage.

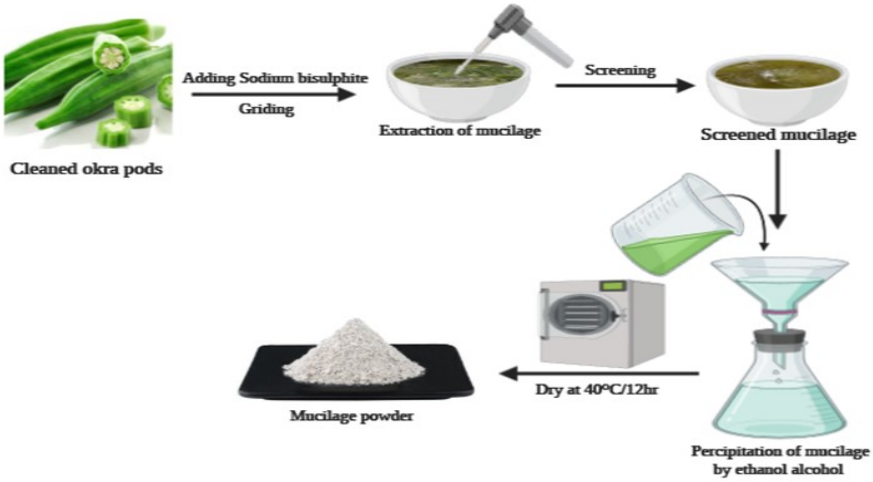

Figure 1. Okra Mucilage Extraction Process

Oleogel preparation

The oleogel (Figure 2) was prepared by melting beeswax at 70 °C until it became clear and transparent. It was then added at a constant concentration of 5% to a mixture of oils comprising 70% palm shortening, 20% olive oil, and 10% flaxseed oil. This oil blend was emulsified using a mixture of emulsifiers, specifically 0.3% soy lecithin and 0.2% monodiglycerides. The emulsified mixture was then heated at 70°C for 30 minutes in a water bath under continuous stirring to ensure homogeneity. After heating, the oleogel was cooled to room temperature (25 °C) without applying shear forces. The samples were subsequently left undisturbed at room temperature for 24 hours to allow proper gel formation (as shown in Figure 2).

Figure 2. The resulted oleogel after cooling at 5°C for 24h.

Phase Diagram Construction

The system under investigation comprised oleogel, a stabilizing agent (okra mucilage), and whey, forming an oil-in-water emulsion. To assess the phase behavior of this ternary system, various concentrations of oleogel and stabilizer were prepared, with whey added to complete the mixture to a total of 100g. Thus, the three components represented in the phase triangle were oleogel, stabilizer, and whey.

Preparation of Cacao Whey Beverages (CWBs)

The formulation of cacao whey beverages (CWBs) is detailed in Table 1. The selected ratios of oleogel and stabilizer to whey were based on the emulsion stability region, in accordance with the method described by Ushikubo & Cunha (2014). As outlined in Table S1, the oleogel concentration increased incrementally by 2.5%, ranging from 2.5% to 12.5%, while the stabilizer concentration increased by 0.1%, from 0.1% to 0.5%. Five groups of CWBs were prepared, each containing different oleogel concentrations (2.5% to 12.5%) combined with a fixed stabilizer concentration. Clockwise increments on the phase diagram represented calculated concentrations of oleogel, stabilizer, and whey, with each point corresponding to a specific formulation (Figure S1). Stability transitions, such as sedimentation, were used to identify boundary samples and delineate the emulsion stability regions on the phase diagram.

Based on these findings, four CWB treatments were prepared:

- CWB1: 5% oleogel + 0.2% okra mucilage
- CWB2: 10% oleogel + 0.2% okra mucilage
- CWB3: 5% oleogel + 0.3% okra mucilage
- CWB4: 10% oleogel + 0.3% okra mucilage
- A control sample (CWB0) was also prepared without the addition of oleogel or okra mucilage.

The pH of the fresh acid whey was adjusted to 6.6 using food-grade NaOH.

Table S1. Formulation that studied to select the ratio of whey, oleogel and stabilizer

SN	Materials of emulsions %	Oleogel	Stabilizer	Whey	O/S Ratio	Stability Index (%)
1	1	2.5	0.1	97.4	25	26.29
2	1	5	0.1	94.9	50	17.17
3	1	7.5	0.1	92.4	75	20.05
4	1	10	0.1	89.9	100	23.93
5	1	12.5	0.1	87.4	125	25.81
6	2	2.5	0.2	97.3	13	19.71
7	2	5	0.2	94.8	25	9.05
8	2	7.5	0.2	92.3	38	21.47
9	2	10	0.2	89.8	50	13.08
10	2	12.5	0.2	87.3	63	27.23
11	3	2.5	0.3	97.2	8	17.14
12	3	5	0.3	94.7	17	6.92
13	3	7.5	0.3	92.2	25	22.9
14	3	10	0.3	89.7	33	15.43
15	3	12.5	0.3	87.2	42	28.66
16	4	2.5	0.4	97.1	6	22.57
17	4	5	0.4	94.6	13	21.45
18	4	7.5	0.4	92.1	19	24.33
19	4	10	0.4	89.6	25	27.21
20	4	12.5	0.4	87.1	31	30.09
21	5	2.5	0.5	97	5	22
22	5	5	0.5	94.5	10	22.88
23	5	7.5	0.5	92	15	25.76
24	5	10	0.5	89.5	20	28.64
25	5	12.5	0.5	87	25	31.52

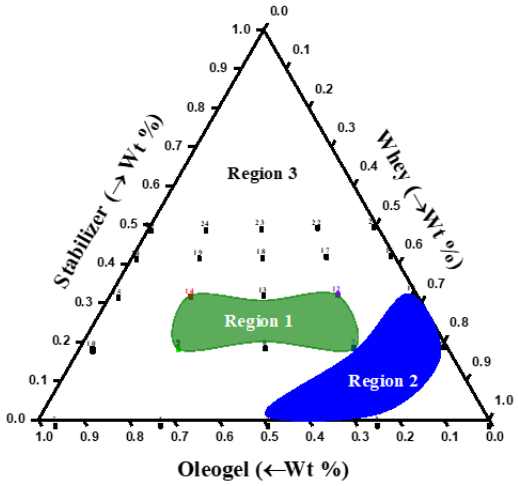

Figure S1. Phase regions for the stability of Cacao whey beverages

The aqueous phase was prepared by adding cacao powder, whey protein isolate (WPI), and sugar (as per Table 1) to the adjusted whey and stirring with a spatula until all components were dissolved. The oil phase was created by heating the oleogel mixture with a portion of fresh whey to 60°C, followed by gradual mixing. The entire mixture was then thermally treated at 85°C for 15 minutes under continuous stirring at 10,000 rpm using a T18 Ultra-Turrax homogenizer (IKA, Königswinter, Germany). After heating, the samples were rapidly cooled in an ice bath for 10 minutes, transferred into 125mL sterilized glass containers, and stored at 5°C until further analysis.

Kinetic stability assessment

The kinetic stability of the emulsions was evaluated by observing phase separation in 25mL transparent cylindrical tubes (17mm internal diameter).

Each tube was sealed with a plastic cap and stored at 25°C for a duration of 14 days. Phase separation was quantified by measuring the Sedimentation Index (SI), calculated as the ratio of the height of the separated oil phase (H) to the initial emulsion height (H_0), according to Equation (1):

$$SI\ (\%) = (H/H0)*100 \qquad (1)$$

In cases where separation of the aqueous phase occurred, the volume percentage of the aqueous phase was also determined relative to the total volume, as per the methodology of Ushikubo & Cunha (2014).

Assessment of the region of CWBs stability

The evaluation of the stability region of CWB emulsions was conducted following a modified protocol based on Ushikubo & Cunha (2014). CWBs were prepared using varying concentrations of oleogel (2.5% to 12.5%, in 2.5% increments) and okra mucilage stabilizer (0.1% to 0.5%, in 0.1% increments), with adjusted whey added to complete the formulations to 100g, as detailed in Table S1. Each CWB group consisted of five samples, all having fixed stabilizer concentrations and varying oleogel percentages. Transitioning from one formulation point to another followed a clockwise trajectory on the phase diagram, representing a row of three-component concentrations (oleogel, stabilizer, and whey) as shown in Figure S1. Stability changes, such as visible sedimentation or phase separation between successive samples, were used to identify boundary points, and these were plotted to define the emulsion stability region. The binodal curve in Figure S2 represents the phase behavior of the CWB formulations, demarcating the boundary between one-phase (stable emulsion) and two-phase (phase-separated) systems. This phase boundary is crucial for understanding the physical and functional stability of CWB formulations. Similar trends have been observed in previous research on whey protein-based emulsions, where stabilizers such as polysaccharides shifted the binodal curve, improving emulsion stability (Nicolai & Murray, 2017; Yvonne & Jideani, 2018; Yu et al., 2022). These findings support the present study, where a plant derived stabilizer (okra mucilage) was utilized to enhance the stability of the protein–polysaccharide complex in cacao whey beverages.

Table 1. Formulations of cacao whey beverages (CWBs) enriched with oleogel-structured emulsion with protein-polysaccharide complex

NO.	Ingredient	CWB0	CWB1	CWB2	CWB3	CWB4
1	Adjusted Whey	82.50	75.80	70.80	75.70	70.70
2	Oleogel (Fat source)	00.00	05.00	10.00	05.00	10.00
3	Mixture of emulsifiers	00.50	00.50	00.50	00.50	00.50
4	Cacao powder	05.00	05.00	05.00	05.00	05.00
5	Whey protein isolate	01.50	01.50	01.50	01.50	01.50
7	Plant stabilizer (Okra mucilage)	00.00	00.20	00.20	00.30	00.30
8	Sugar	12.00	12.00	12.00	12.00	12.00

CWB0: Adjusted whey without adding oleogel and okra mucilage; CWB1: Formulation with 5% oleogel and 0.2% mucilage; CWB2: Formulation with 10% oleogel and 0.2% mucilage; CWB3: Formulation with 5% oleogel and 0.3% mucilage; CWB4: Formulation with 10% oleogel and 0.3% mucilage.

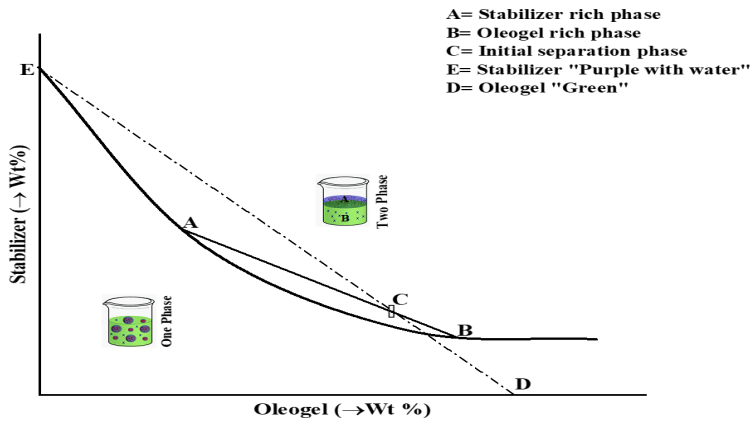

Figure S2. The binodal curve of cacao whey beverages formulation

Determination of Fatty Acids

Fatty acid methyl esters (FAMEs) were synthesized by transesterification of oil samples using sodium methoxide as a catalyst, according to the AOCS Official Method Ce 1–62 (AOCS, 2002). In this procedure, 100mg (±0.5mg) of oil was accurately transferred into a screw-cap vial using a Pasteur pipette. To this, 5 mL of hexane was added, and the mixture was vortexed briefly. Subsequently, 250µL of sodium methoxide solution was introduced, followed by vortexing for one minute. Afterward, 5mL of saturated sodium chloride solution was added, the vial was sealed, and the contents were vigorously shaken for 15 seconds before being left undisturbed for 10 minutes. The upper hexane layer was carefully collected and dried over anhydrous sodium sulfate (Na_2SO_4) for 20 minutes. The treated hexane layer containing FAMEs was then injected into a gas chromatography system (Agilent 6890N Network GC-System, Agilent Technologies, Wilmington, USA) equipped with an auto-sampler (Agilent 7683 Series), an injector (Agilent 7683-B Series), and a flame ionization detector (FID). Separation was achieved using a DB-Wax capillary column (30m length × 0.30mm internal diameter), with helium as the carrier gas at a flow rate of 1mL/min. A 1µL sample was injected using a split ratio of 1:20. The injector and detector temperatures were set at 250°C. The oven temperature program was as follows: an initial temperature of 100 °C held for 2 minutes, ramped at 5°C/min to 230°C, then held at 230°C for 10 minutes. Identification of individual FAMEs was conducted by comparing retention times with those of a standard mixture (37-Component FAME Mix, SUPELCO). Fatty acid content was expressed as the percentage by weight of total identified fatty acids.

Nutritional Indices and Fatty Acid Ratios

The atherogenic index (AI) and thrombogenic index (TI) were calculated according to the formulas proposed by Ulbricht & Southgate (1991):

AI=C12:0+(4*C14:0)+C16:0/n-6PUFA+n-PUFA+ MUFA

TI=C14:0+C16:0+C18:0/(0.5*MUFA)+(0.5*n-6PUFA)+ (3*n-3PUFA)

PUFA= Polyunsaturated fatty acids;

MUFA = Monounsaturated fatty acids

Viscosity

The apparent viscosity of the samples was measured directly using a Brookfield Digital Rheometer (HA DVIII Ultra, Brookfield Engineering Laboratories Inc., Massachusetts, United States). A 25mL sample was transferred into a 50mL container (cup radius: 15mm), and the HA-05 spindle was selected for measurement. Viscosity was recorded over a shear rate range of 19 to 578s^{-1} for a duration of 1.80 minutes, comprising 10 measurement intervals, each lasting 10 seconds, conducted at a temperature of 25°C. The viscosities reported in this study were specifically assessed at a shear rate of 100s^{-1}.

Determination of z-average hydrodynamic diameter and ζ-potential of WB-emulsion

Particle size was determined by dynamic light scattering (DLS) using a Nano Sizer ZS (Malvern Instruments, UK), with measurements conducted at 25°C. Particle size was characterized using the z-average and mean diameter derived from volume distribution. The z-average refers to the intensity-weighted mean hydrodynamic diameter, as determined by DLS. Volume distribution is especially important when the measured intensity distribution is significantly influenced by the presence of larger particles. The polydispersity index (PDI) was calculated as the ratio between the squared and linear coefficients obtained from the cumulant analysis. The ζ-potential of the WB-emulsion was determined via light scattering techniques combined with the application of an alternating electric field to the measurement cell, using a protein concentration of 2% (w/v). Dispersions were introduced into the electrophoretic mobility cell and examined at a scattering angle of 173 degrees using the Nano Sizer ZS. The applied electric field ranged from -200 to 200V, depending on the conductivity of the samples. The overall electrophoretic mobility of the particles was measured, and the corresponding ζ-potential (V) was calculated using the Smoluchowski equation (Motalebi Moghanjougi et al., 2021).

Microstructure of beverages

The microstructure of the samples was examined following the method of Moslehishad & Ezzatpanah (2010), using high-resolution transmission electron microscopy (HR-TEM, Tecnai G20, FEI, Netherlands). Samples were prepared by mixing glutaraldehyde with whey beverages at a 1:7 (v/v) ratio, followed by dilution at 1:50 using 0.01M calcium chloride. For staining, a drop of the diluted sample was placed onto formvar-coated TEM grids and allowed to incubate for one minute. A drop of 2% phosphotungstic acid (pH 7.2) was then added. The grids were air-dried prior to imaging. TEM imaging was performed at an accelerating voltage of 100 kV and a magnification of 200,000×.

Microbiological analysis

Total plate counts were estimated using the pour plate method in accordance with the International Organization for Standardization (ISO, 2013). Plates containing total plate count agar were incubated at 30 °C for 72 hours. Yeast and mold counts were assessed using yeast peptone dextrose agar (Oxoid), supplemented with 0.1g/L chloramphenicol (Oxoid), and incubated at 25°C for 3–5 days (Jay et al., 2005). Coliform bacteria were enumerated on Violet Red Bile (VRB) agar (pH 7.0–7.2), and incubated at 37°C for 24–48 hours. Colonies displaying pink to red-purple coloration, with or without surrounding halos of precipitation, were identified as coliforms (Atlas, 2004). All determinations were performed in triplicate, and results were expressed as log cfu/mL.

Statistical analysis

The General Linear Model (GLM) was employed to perform a one-way analysis of variance to assess the differences in means across various groups. Subsequent post hoc multiple comparisons were executed utilizing Tukey's test, with a statistical significance threshold set at $P \leq 0.05$, as analyzed using SAS (version 9.4 TS Level 1M3, SAS Institute Inc., Cary, NC, USA). Throughout the analysis, least squares means were computed and reported. The data displayed in the tables represents the mean of three measurements along with the (± standard deviation).

3. Results and Discussions

Physiochemical composition of fresh cacao whey beverages

The Cacao-flavored whey beverages presented the following proximate composition (g/100mL, Table 2): TS (23.30±0.11-33.68±0.21), fat (0.89± 0.03-10.91±0.06), total carbohydrates (15.46±0.18-16.25±0.10), protein (2.94±0.05-3.48±0.18), and ash (1.09±0.12-1.46±0.03). Incorporation of oleogel compound to beverage significantly increased total solids content ($P \leq 0.05$) of the treatments. Significant differences ($P \leq 0.05$) were observed for fat, total carbohydrates, protein, and ash levels for the whey beverages treatments. The pH values were in the range (6.39±0.09-6.53±0.05). No significant changes ($P \geq 0.05$) were observed for pH values.

Table 2. The physicochemical compositions of fresh cacao whey beverages (CWBs) enriched with oleogel-structured emulsion with protein-polysaccharide complex

Parameters	Cacao whey beverages				
	CWB0	CWB1	CWB2	CWB3	CWB4
Total solids (g/100 mL)	23.30±0.11^{c}	28.60±0.10^{b}	33.57±0.14^{a}	28.79±0.10^{b}	33.68± 0.21^{a}
Fat (g/100 mL)	0.89±0.03^{c}	5.89±0.02^{b}	10.89±0.10^{a}	5.94±0.04^{b}	10.91±0.06^{a}
Total carbohydrates (g/100 mL)	15.46±0.18^{c}	15.91±0.10^{b}	15.98±0.12^{ab}	16.12±0.11^{ab}	16.25±0.10^{a}
Protein (g/100 mL)	2.94±0.05^{b}	3.21±0.10^{ab}	3.34±0.19^{a}	3.37± 0.04^{a}	3.48±0.18^{a}
Ash (g/100 mL)	1.09±0.12^{c}	1.11±0.12^{bc}	1.25±0.06^{abc}	1.32±0.02^{ab}	1.46±0.03^{a}
pH	6.53±0.05^{a}	6.39±0.09^{a}	6.45±0.06^{a}	6.42±0.06^{a}	6.49±0.09^{a}

CWB0: Adjusted whey without adding oleogel and okra mucilage; **CWB1:** Formulation with 5% oleogel and 0.2% mucilage; **CWB2:** Formulation with 10% oleogel and 0.2% mucilage; **CWB3:** Formulation with 5% oleogel and 0.3% mucilage; **CWB4:** Formulation with 10% oleogel and 0.3% mucilage. Small superscript letters in the same raw indicate a significant difference in means at $P \leq 0.05$. All values are presented as means ±SD, n = 3.

Fatty acid composition of cacao whey beverages (CWBs)

The fatty acid profile of the CWBs illustrated in Table 3 reveals significant differences ($P \leq 0.05$) among the various treatments, indicating that the composition of the oleogel used in the manufacture of CWBs had a profound impact on the fatty acid composition. A notable observation is the marked increase in the concentration of unsaturated fatty acids (USFA) and polyunsaturated fatty acids (PUFA) in all the CWB treatments compared to the control. This outcome is desirable, as increased USFA and PUFA are linked to numerous health advantages. The results show that the samples with a higher fat content (CWB2 and CWB4) had a greater proportion of unsaturated fatty acids, particularly monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), compared to the lower fat samples (CWB1 and CWB3). The increased presence of MUFAs and PUFAs in the higher fat CWB formulations can be attributed to the specific composition of the oleogels used in their preparation. Oleogels are known to effectively reduce the levels of saturated and trans fatty acids while increasing the ratio of healthier unsaturated fatty acids (Goyal et al., 2015; Martins et al., 2018; Jagtap et al., 2020). The incorporation of flaxseed oil and olive oil in the oleogel fractions seems to be the primary driver for the observed increase in MUFAs and PUFA. Flaxseed oil is a substantial source of alpha-linolenic acid (ALA), an omega-3 fatty acid recognized for its anti-inflammatory effects and its ability to reduce blood lipid levels (Gambino et al., 2018; Bordón et al., 2019; Aziz et al., 2024). Additionally, extra virgin olive oil is known for its high amount of monounsaturated fatty acids, especially oleic acid, which has been associated with cardioprotective effects (Oliveira et al., 2024). Conversely, the content of saturated fatty acids (SFA), such as palmitic acid (C16:0), decreased significantly in the modified CWB treatments compared to the control. This is a positive development, as high intakes of SFA have been associated with a heightened risk of cardiovascular disease (Edel et al., 2015; Sharma & Ramanathan, 2023). The changes in the fatty acid profile are also reflected in the improved nutritional indices, such as the atherogenic index (AI) and the thrombogenic index (TI), which were significantly reduced in the modified CWB treatments. These indices are important indicators of the potential for the development of cardiovascular diseases, and their reduction suggests an improved nutritional quality of the CWBs (Shin et al., 2021 and Gao et al., 2022). Furthermore, the incorporation of the oleogel fractions led to a beneficial equilibrium between omega-6 and omega-3 fatty acids, which is essential for maintaining a healthy inflammatory response and overall well-being (Edel et al., 2015; Sharma & Ramanathan, 2023). The omega-6 to omega-3 ratio in the modified CWBs falls within the recommended range, further highlighting the potential health benefits of the reformulated products. Based on the information provided in Table 3, the omega-6 to omega-3 ratio (Omega) for the different CWB treatments appears to be in line with the findings from the review by Simopoulos, (2016); Tang et al., (2020); Sharma & Ramanathan, (2023). Table 3 shows the following Omega values for the CWB treatments. These values indicate that the modified CWB treatments have a significantly lower omega-6 to omega-3 ratio compared to the control. Specifically:

- The control sample has an omega-6 to omega-3 ratio of 48.87, which is much higher than the recommended range of 1:1 to 4:1.
- The modified CWB treatments have ratios ranging from 24.43 to 31.15, which fall within the recommended range.

This suggests that the incorporation of the oleogel fractions containing flaxseed oil and extra virgin olive oil has effectively reduced the ratio of omega-6 to omega-3 in the modified CWBs, bringing it closer to the optimal range for maintaining brain and gut health, as per the findings from Simopoulos (2016); Tang et al. (2020); Shin et al. (2021); Sharma & Ramanathan (2023). The reduced ratio of omega-6 to omega-3 in the modified CWBs is a positive outcome and aligns with the discussions on the improved fatty acid composition and nutritional parameters observed in the provided results. Furthermore, the incorporation of oleogel in the CWB formulations provides an opportunity to deliver bioactive lipophilic

compounds, such as carotenoids or fat soluble vitamins, in a more stable and controlled manner (Martins et al., 2018). This can improve the overall nutritional quality and functionality of the cacao-whey beverages. In summary, the analysis of the composition of fatty acids highlights the potential of using oleogels in the development of cacao-whey beverages, as they can contribute to a more favorable mouth-feel and nutritional profile by increasing the ratio of unsaturated fatty acids while providing a platform for the delivery of lipophilic bioactive compounds. Vitamins, in a more stable and controlled manner (Martins et al., 2018). This can improve the overall nutritional quality and functionality of the cacao-whey beverages.

Table 3. Fatty acid composition of cacao whey beverages (CWBs) enriched with oleogel-structured emulsion with protein- polysaccharide complex

*Fatty acids	CWB0	CWB1	CWB2	CWB3	CWB4
C6:0	0^{a}	0.1 ± 0.02^{a}	0.24 ± 0.04^{a}	0.21 ± 0.05^{a}	0.38 ± 0.90^{a}
C8:0	0^{a}	0.11 ± 0.06^{a}	0.16 ± 0.07^{a}	0.13 ± 0.03^{a}	0.39 ± 0.88^{a}
C10:0	0^{c}	0.11 ± 0.05^{b}	0.14 ± 0.07^{ab}	0.13 ± 0.03^{ab}	0.15 ± 0.03^{a}
C12:0	0.21 ± 0.04^{d}	1.28 ± 0.06^{b}	1.24 ± 0.07^{bc}	1.44 ± 0.17^{a}	1.18 ± 0.08^{c}
C14:0	1.13 ± 0.12^{c}	1.49 ± 0.05^{a}	1.23 ± 0.22^{bc}	1.50 ± 0.13^{a}	1.30 ± 0.07^{b}
C15:0	0.10 ± 0.03^{a}	0.05 ± 0.05^{b}	0.07 ± 0.02^{ab}	0.06 ± 0.11	0.10 ± 0.03^{a}
C16:0	51.32 ± 0.35^{a}	35.23 ± 0.04^{c}	35.85 ± 0.08^{bc}	35.85 ± 1.74^{bc}	36.45 ± 0.88^{b}
C16:1	0.13 ± 0.09^{c}	0.22 ± 0.07^{b}	0.29 ± 0.10^{ab}	0.28 ± 0.09^{ab}	0.34 ± 0.06^{a}
C17:0	0.12 ± 0.05^{a}	0.12 ± 0.05^{a}	0.13 ± 0.04^{a}	0.13 ± 0.09^{a}	0.16 ± 0.06^{a}
C17:1	0.02 ± 0.02^{b}	0.04 ± 0.11^{ab}	0.04 ± 0.06^{ab}	0.03 ± 0.03^{ab}	0.06 ± 0.05^{a}
C18:0	4.66 ± 0.16^{c}	6.44 ± 0.22^{b}	6.82 ± 0.07^{a}	6.45 ± 0.08^{b}	6.92 ± 0.17^{a}
C18:1	33.62 ± 0.08^{b}	39.26 ± 0.05^{a}	39.63 ± 1.21^{a}	39.36 ± 0.05^{a}	39.68 ± 0.93^{a}
C18:2T	0.17 ± 0.02^{a}	0^{b}	0^{b}	0^{b}	0^{b}
C18:2C	7.96 ± 0.09^{c}	13.44 ± 0.05^{b}	14.16 ± 0.90^{a}	13.64 ± 0.07^{b}	14.16 ± 0.90^{a}
C18:3C	0.16 ± 0.03^{c}	0.44 ± 0.12^{b}	0.53 ± 0.10^{ab}	0.45 ± 0.10^{ab}	0.55 ± 0.12^{a}
C20:0	0.30 ± 0.07^{b}	0.38 ± 0.10^{ab}	0.42 ± 0.05^{a}	0.39 ± 0.06^{ab}	0.43 ± 0.14^{a}
C20:1	0.12 ± 0.03^{c}	0.34 ± 0.05^{b}	0.38 ± 0.09^{b}	0.38 ± 0.05^{b}	0.48 ± 0.09^{a}
C22:0	0^{c}	0.11 ± 0.04^{b}	0.18 ± 0.05^{a}	0.17 ± 0.05^{ab}	0.19 ± 0.12^{a}
SCFAs	0^{c}	0.33 ± 0.06^{bc}	0.54 ± 0.08^{b}	0.45 ± 0.14^{b}	0.92 ± 0.84^{a}
MCFAs	1.39 ± 0.21^{c}	2.54 ± 0.28^{b}	2.84 ± 0.08^{a}	2.58 ± 0.10^{b}	3.04 ± 0.37^{a}
SFAs	56.41 ± 0.25^{a}	42.76 ± 0.15^{c}	43.48 ± 1.66^{bc}	42.93 ± 0.21^{bc}	43.66 ± 0.77^{b}
USFAs	42.21 ± 0.09^{c}	54.10 ± 0.29^{b}	54.41 ± 0.13^{b}	54.16 ± 0.50^{b}	55.27 ± 0.87^{a}
MUFAs	33.88 ± 0.7^{b}	39.96 ± 0.27^{a}	40.39 ± 1.12^{a}	40.04 ± 0.13^{a}	40.45 ± 0.88^{a}
PUFAs	8.24±0.15c	13.78 ± 0.76^{b}	14.09 ± 0.19^{b}	13.90 ± 0.15^{b}	14.69 ± 0.81^{a}
TFA	0.12 ± 0.02^{a}	0^{b}	0^{b}	0^{b}	0^{b}
Omega FAs	48.87 ± 8.34^{a}	24.43 ± 6.51^{b}	29.90 ± 6.42^{b}	27.10 ± 6.80^{b}	31.15 ± 8.14^{b}
AI	1.33 ± 0.00^{a}	0.78 ± 0.03^{b}	0.79 ± 0.00^{b}	0.78 ± 0.04^{b}	0.79 ± 0.02^{b}
TI	9.86 ± 0.09^{a}	5.26 ± 0.29^{b}	5.37 ± 0.09^{b}	5.31 ± 0.05^{b}	5.39 ± 0.18^{b}

CWB0: Adjusted whey without adding oleogel and okra mucilage; **CWB1:** Formulation with 5% oleogel and 0.2% mucilage; **CWB2:** Formulation with 10% oleogel and 0.2% mucilage; **CWB3:** Formulation with 5% oleogel and 0.3% mucilage; **CWB4:** Formulation with 10% oleogel and 0.3% mucilage. Small superscript letters indicate a significant difference in means at $P \leq 0.05$. All values are presented as means ±SD, n = 3. *(%) fat content is the percent of total fat in the sample. **SCFAs:** Short chain fatty acids; **MCFAs:** Medium chain fatty acids; **SFAs:** Saturated fatty acids; **USFAs:** Unsaturated fatty acids; **MUFAs:** Monounsaturated fatty acids; **PUFAs:** Polyunsaturated fatty acids; **TFA:** Trans fatty acids; **Omega FAs:** Omega fatty acids; **AI:** Atherogenic index; **TI:** Thrombogenic index.

Viscosity

The rheological properties, particularly viscosity, are critical quality attributes that directly influence the functionality and sensory acceptability of various food and beverage applications. The results presented in this study demonstrate that the incorporation of different oleogel and okra mucilage levels significantly impacted the viscosity profiles of the CWB formulations. Compared to the control sample (CWB0) , the treatments (CWB1, CWB2, CWB3, CWB4) exhibited a marked increase in viscosity as shown in Figure 3. This observation can be attributed to the synergistic effects of the structural and compositional changes induced by the incorporation of both the oleogel components and the okra mucilage. As shown in Figure 3, the results indicated that increasing the concentration of okra mucilage from 0.2% to 0.3% significantly increased the continuous phase viscosity of the emulsions (CWB1 vs CWB3, CWB2 vs CWB4). This is attributed to the inherent viscous nature of the biopolymer mucilage, even at low concentrations. The high water-holding capacity of okra mucilage, due to its polysaccharide composition of rhamnose, galacturonic acid, galactose, glucose, and glucuronic acid, contributes to the enhanced viscosity (Zhu & Obara, 2022; Tudu & Samanta, 2023; Fatima et al., 2024). Also, the synergetic interaction of fatty acids could impact the structure and attributes of the system. Pedersen et al., (2023) noted that lowering the fat concentration in chocolate milk beverages led to a reduction in viscosity. As increased viscosity in the samples is associated with the presence of high-melting point saturated fatty acids, including palmitic (C16:0) and stearic (C18:0) acids, within the oleogel fractions. These saturated fatty acids contribute to the formation of a more structured and rigid network, leading to an enhanced viscosity of beverage systems (Patel & Dewettinck, 2015; Martín-Alfonso et al., 2022; Souza et al., 2024). Furthermore, the inclusion of medium chain fatty acids (MCFAs), such as caprylic (C8:0) and capric (C10:0) acids, in the oleogel fractions may have also contributed to the increase in the viscosity of the CWBs that are enriched with oleogel structured emulsion with protein polysaccharide complex. As MCFAs are known to have lower melting points and can contribute to the plasticity and mouth-feel characteristics of the beverages (Patel & Dewettinck, 2015; Meissner et al., 2020; Plazzotta et al., 2023; Souza et al., 2024). Additionally, the up-mode shear rate tests revealed shear-thickening behavior for the CWB1 and CWB2 samples, where the apparent viscosity exhibited an increase as the shear rate was elevated. This is likely due to the ability of the mucilage biopolymer to enhance solution viscosity through its interactions with the whey protein network. The formation of a stronger protein network, potentially through hydrogen bonding or increased water retention by mucilage, may have contributed to this shear-thickening phenomenon (Wijekoon et al., 2023; Fatima et al., 2024; Raj et al., 2024). In contrast, the CWB3 and CWB4 samples, with a more pseudoplastic, non-Newtonian flow behavior, where the apparent viscosity diminished as the shear rate increased. This suggests that the higher mucilage concentration led to a more structured, gel-like continuous phase that aligned and disentangled under shear, resulting in a shear-thinning response. It is worth noting that the viscosity of CWB1 and CWB2, with the lower mucilage content, was quite similar at higher shear rates, indicating that the fat content (5% vs. 10%) had a relatively minor influence on the overall viscosity compared to the mucilage concentration. This suggests that the mucilage was the predominant factor governing the rheological properties of the CWB formulations. The control sample (CWB0) exhibited a lower viscosity profile compared to other treatments, highlighting the significant impact of the added mucilage and fat on the rheological behavior of the cacao whey beverages. These results add to the expanding body of research regarding the utilization of dairy byproducts, such as whey, and the incorporation of functional ingredients like polysaccharides and oleogels to develop innovative and stable beverage products (Martins et al., 2016).

Overall, the viscosity of the cacao-whey beverages was strongly influenced by the concentration of okra mucilage, which acted as a potent thickening and structuring agent. The higher mucilage content led to increased continuous phase viscosity and a shift from shear-thickening to shear-thinning behavior, likely due to the formation of a more structured, gel-like

network. Moreover, the fat content played a secondary role, with the mucilage being the primary determinant of the rheological characteristics of the CWB formulations. The increased viscosity observed in the CWBs can be advantageous for different foods and beverage applications, as it can improve the texture, stability, and sensory properties of the final products. For instance, the higher viscosity can contribute to a more desirable consistency and mouthfeel in chocolate-flavored beverages, leading to improved consumer acceptance and extended shelf-life.

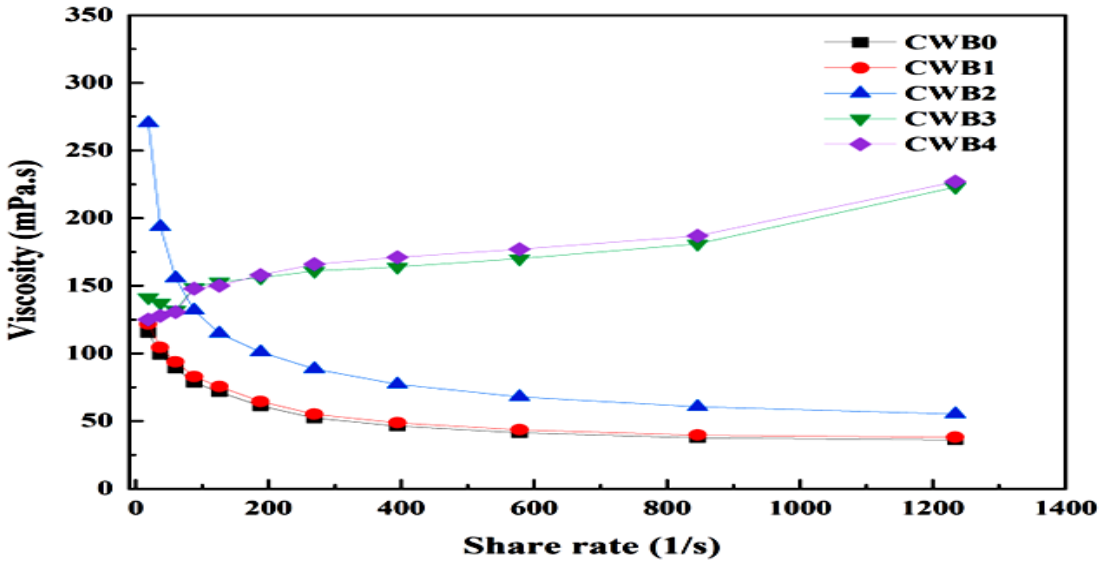

Figure 3. Apparent viscosity as a function of shear rate for Cacao whey beverages (CWBs)

CWB0: Adjusted whey without adding oleogel and okra mucilage; **CWB1:** Formulation with 5% oleogel and 0.2% mucilage; **CWB2:** Formulation with 10% oleogel and 0.2% mucilage; **CWB3:** Formulation with 5% oleogel and 0.3% mucilage; **CWB4:** Formulation with 10% oleogel and 0.3% mucilage.

The particle size, polydispersity index (PDI) and ζ-Potential values

As presented in Table 4, the particle size distribution of the CWB formulations ranged from 224.9nm to 354.2nm, indicating a narrow distribution within the nanometric to submicron range (McClements & Rao, 2011). This particle size range is desirable, as it enhances the mouthfeel, creaminess, and visual appeal of the final beverage. Among the treatments, CWB3 exhibited the smallest average particle size (224.9 nm), while CWB1 showed the largest (354.2 nm).

The polydispersity index (PDI) values ranged from 0.35 to 0.44, suggesting relatively narrow size distributions for all formulations (Piorkowski & McClements, 2014). The lower PDI and smaller particle size observed in CWB3 and CWB4 can be attributed to their higher content of okra mucilage, which is rich in polysaccharides. These polysaccharides can adsorb onto droplet surfaces, enhancing electrostatic and steric stabilization and thereby preventing droplet aggregation (Deters et al., 2005; Al-Sayed et al., 2012). In contrast, CWB1 and CWB2, which had lower mucilage content, exhibited larger particle sizes and higher PDIs. This may indicate more heterogeneous droplet populations, likely due to weaker interfacial stabilization. The oleogel components, comprising various saturated, monounsaturated, and polyunsaturated fatty acids (from flaxseed oil and extra virgin olive oil), play a crucial role in forming the emulsion network and determining interfacial characteristics (Lopez-Martínez et al., 2015; Patel & Dewettinck, 2015). The presence of saturated fatty acids notably palmitic acid (C16:0) and stearic acid (C18:0) in the oleogel can promote the formation of a more rigid and organized emulsion structure, which may result in larger, less uniform particles. This effect, especially when combined with beeswax and reduced mucilage, likely explains the higher particle size and PDI values in CWB1 and CWB2. On the other hand, monounsaturated fatty acids (oleic acid, C18:1) and polyunsaturated fatty acids (linoleic acid, C18:2; alpha-linolenic acid, C18:3) enhance fluidity,

leading to the formation of smaller and more uniform particles, as observed in CWB3 and CWB4. The zeta potential (ζ-potential) values of the CWBs ranged from −5.24mV to −28.7mV, suggesting that the emulsions were electrostatically stabilized (Piorkowski & McClements, 2014). CWB3 recorded the highest negative zeta potential (−28.7mV), indicating a stronger surface charge on the dispersed particles. This can improve repulsion between particles, thereby enhancing stability against aggregation and phase separation. The high zeta potential values observed in CWB3 and CWB4 are consistent with their elevated mucilage content. The polysaccharides present in the mucilage likely adsorbed onto the droplet surfaces, increasing the surface charge and reinforcing electrostatic stabilization (Dickinson, 1992; Friberg et al., 2003; McClements, 2004). Combined with their small particle sizes and low PDIs, this indicates that CWB3 and CWB4 possess superior emulsion stability compared to CWB1 and CWB2. Furthermore, the interactions between the oleogel fatty acid profile and beeswax can influence particle surface properties and charge. The adsorption of oleogel and beeswax components onto the droplet surfaces may modify interfacial characteristics, as reflected in the ζ-potential values (Patel & Dewettinck, 2015; Martins et al., 2016). In the case of CWB3 and CWB4, the stabilizing effect of mucilage appears to dominate, potentially overshadowing the influence of the oleogel composition. The electrosteric stabilization provided by mucilage polysaccharides contributed to their smaller particle sizes, narrower distributions, and higher zeta potentials. In summary, the particle size distribution, PDI, and zeta potential data highlight the critical role of mucilage and oleogel composition in determining the physicochemical stability of the cacao whey beverages. Further research into the interfacial interactions between whey proteins, mucilage polysaccharides, oleogel lipids, and beeswax could offer deeper insight into the mechanisms behind the observed emulsion behaviors.

Table 4. Particle size, PDI and ζ- potential of aqueous phase, oil phase and cacao whey beverages (CWBS) enriched with oleogel-structured emulsion with protein-polysaccharide complex

	Aqueous phase	Oil phase	CWB 1	CWB 2	CWB 3	CWB 4
Size (d.nm)	202.9±0.4^{e}	50±0.08^{f}	354.2±0.4^{a}	327.5±0.60^{b}	224.9±0.60^{d}	314.6±0.60^{c}
PDI	0.34±0.03^{d}	0.51±0.02^{a}	0.35±0.03^{d}	0.39±0.02^{c}	0.44±0.02^{b}	0.39±0.02^{c}
ζ- potential (mV)	- 24.7±0.20^{c}	- 65.3±0.40^{e}	- 5.24±0.12^{a}	-18.60±0.10^{b}	- 28.70±0.10^{d}	- 24.70±0.10^{c}

CWB0: Adjusted whey without adding oleogel and okra mucilage; **CWB1:** Formulation with 5% oleogel and 0.2% mucilage; **CWB2:** Formulation with 10% oleogel and 0.2% mucilage; **CWB3:** Formulation with 5% oleogel and 0.3% mucilage; **CWB4:** Formulation with 10% Oleogel and 0.3% mucilage. Small superscript letters indicate a significant difference in means at P ≤0.05. All values are presented as means ±SD, n = 3

Microstructural Analysis of Cacao Whey Beverages Using Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) provided valuable insights into the microstructure of the various components and formulations of the cacao whey beverages (CWB). The TEM micrograph of the aqueous phase (Figure 4A) showed a homogeneous dispersion, lacking any distinct structural entities, suggesting a continuous matrix composed of whey proteins, okra mucilage polysaccharides, sugars, and cocoa powder. This observation is consistent with Wijaya et al. (2017), who reported similar dispersion of proteins and polysaccharides in oil-in-water emulsion systems. In contrast, the TEM image of the oil phase (Figure 4B) revealed a clear core-shell structure, where the core consisted of oleogel (a mixture of beeswax, shortening, olive oil, and flaxseed oil), surrounded by an interfacial layer. This structural organization can be attributed to the self-assembly of amphiphilic oleogelator components, such as beeswax and lecithin, which orient at the oil-water interface to stabilize the oleogel network (Wang et al., 2024). Similar structural arrangements have been observed in other oleogel-based emulsions (Park et al., 2022; Huang et al., 2023), underscoring the crucial role of oleogels in oil phase stabilization. The TEM images of CWB1 and CWB2 (Figures 4C and D), which contain 0.2% mucilage and either 5% or 10% oleogel, respectively, showed relatively larger and more heterogeneous droplets.

This heterogeneity suggests weaker stabilization of the oil droplets in these samples due to the lower mucilage concentration. Conversely, the CWB3 and CWB4 formulations (Figures 4E and F), which contain 0.3% mucilage, demonstrated a more homogeneous and finely dispersed microstructure. The increased mucilage content likely promoted better stabilization via polysaccharide adsorption at the interface and the formation of a polysaccharide-protein network in the aqueous phase (Raj et al., 2020; Yue et al., 2024). The TEM micrographs of CWB3 and CWB4 revealed well-defined core-shell structures, where the oleogel core was encased within a distinct interfacial layer composed of adsorbed whey proteins and mucilage polysaccharides. This multilayered interfacial architecture likely contributed to their improved physical stability, as supported by previous findings (Huang et al., 2023; Plazzotta et al., 2023). Similar observations were made by Huang et al. (2023), who reported enhanced emulsion stability through the use of polysaccharides such as pectin in combination with whey proteins to form core-shell structures in oleogel-based systems. The formation of such a core-shell structure can be attributed to synergistic interactions between the emulsion components. Whey proteins initially adsorb at the oil-water interface, followed by the interaction of mucilage polysaccharides with these adsorbed proteins, forming a protective interfacial network. This protein polysaccharide complex helps prevent droplet coalescence and aggregation, thereby enhancing overall emulsion stability (Wijaya et al., 2017; Park et al., 2022). These findings are in agreement with studies that have shown how interactions between whey proteins and oleogel components improve the mechanical properties and stability of oleocolloid systems (Park et al., 2022). The enhanced physical stability observed in CWB3 and CWB4 suggests that these formulations hold promise for use in functional food and beverage applications, as the core-shell structure and stabilized interfacial layer could improve not only stability but also mouthfeel and sensory quality. In summary, the TEM analysis revealed critical microstructural differences among the CWB formulations. These differences emphasize the pivotal role of mucilage concentration and protein-polysaccharide interactions in forming a robust core-shell structure that stabilizes the oil-in-water emulsion system. The results strongly support the incorporation of mucilage and structured oleogels to enhance the physicochemical performance of cacao whey beverage formulations.

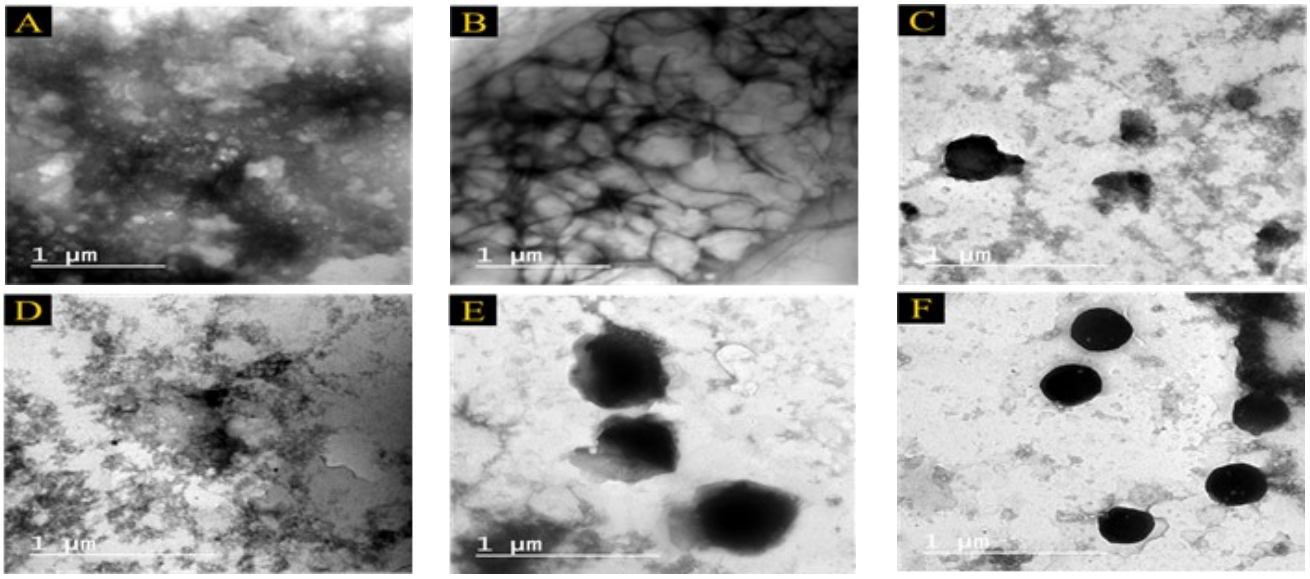

Figure 4. TEM images of Cacao whey beverages (CWBs)

(A) Aqueous phase; **(B) Oil phase; CWB1 (C):** Formulation with 5% oleogel and 0.2% mucilage; **CWB2 (D):** Formulation with 10% oleogel and 0.2% mucilage; **CWB3 (E):** Formulation with 5% oleogel and 0.3% mucilage; **CWB4 (F):** Formulation with 10% oleogel and 0.3% mucilage.

Phase Diagram Analysis and Formulation Optimization

The pseudo-ternary phase diagram is a valuable tool for identifying the optimal formulation window for the stability, texture, and sensory appeal of CWBs. Analyzing the three distinct regions solid-like, saturated, and phase separation provides crucial insights into the interactions between the key ingredients: oleogels and polysaccharides.

Region 1: Solid-like Emulsions

Formulations within this region exhibit highly viscous, stable emulsions due to a narrow particle size distribution. The nanometric-to-submicron droplet sizes contribute significantly to the desirable mouthfeel and visual appeal, resulting in creamy and smooth textures in CWBs. This behavior can be attributed to the synergistic interaction between the gel-like nature of oleogels and the stabilizing effect of polysaccharides, such as okra mucilage. Studies have shown that the self-assembled fibrillar networks (SAFiNs) formed by oleogels provide steric stabilization and enhance the overall texture of emulsions (Sagiri & Poverenov, 2024). The improved stability observed in this region is linked to the optimal balance between oleogel and polysaccharide concentrations. Okra mucilage, for instance, has been reported to serve as an effective emulsifier and stabilizer, promoting gelation and preventing droplet aggregation (Liang et al., 2023). Furthermore, the combination of okra mucilage with xanthan gum or guar gum has been shown to enhance the emulsifying and stabilizing properties of food-based emulsions. The submicron droplet size observed in these CWBs is crucial for preventing phase separation, as smaller droplets improve emulsion stability by limiting coalescence (Bai et al., 2017; McClements et al., 2017; Dantas et al., 2021).

Region 2: Saturated Systems

In Region 2, the emulsions initially appear stable, but gravitational forces (creaming and sedimentation) begin to affect their stability after approximately 14 days. The systems in this region exhibit a delicate balance between oleogel and stabilizer concentrations, leading to viscosity changes over time. This reflects a saturated system where even minor imbalances can lead to phase separation, especially at boundary points where the oleogel-to-polysaccharide ratio is critical. The high oleogel content in this region increases the likelihood of gravitational separation due to the limited ability of polysaccharides to effectively stabilize the dispersed oil phase in highly saturated systems. Previous studies on emulsions have demonstrated that maintaining a balance between stabilizers and gelators is essential for ensuring homogeneity, particularly in high-fat-content beverages (Bai et al., 2018; Du Le et al., 2020). When the concentration of okra mucilage is reduced, the CWBs lose their ability to maintain gel structure, resulting in instability. As in Region 1, the stability of these systems is linked to an optimal balance between oleogel and polysaccharide concentrations. Okra mucilage has been shown to act as an effective emulsifier and stabilizer, promoting gelation and preventing droplet aggregation. Its combination with xanthan gum or guar gum further enhances the emulsifying and stabilizing properties of food-based emulsions. The submicron droplet sizes observed in these CWBs remain essential for preventing phase separation by reducing coalescence (Bai et al., 2017; McClements et al., 2017; Dantas et al., 2021; Liang et al., 2023; Weerapol et al., 2024).

Region 3: Phase Separation

In this region, phase separation occurs in nearly all formulations due to extreme ratios of oleogels and stabilizers. Samples prepared with stabilizer concentrations below 0.2% or above 0.3% (w/w) exhibit rapid phase separation and precipitation. This observation aligns with previous research showing that improper emulsifier concentrations can lead to phase separation, droplet aggregation, and eventual sedimentation in similar systems (Timms, 1984; Martins et al., 2018; Manzoor et al., 2022; Sagiri & Poverenov, 2024). The presence of insoluble aggregates at the bottom and a cloudy serum layer at the top in these unstable formulations highlights the necessity for precise control over the oil-to-water ratio and stabilizer concentration. Studies on similar oleogel-based systems suggest that such phase separation often results from the inability of gel networks to stabilize droplets

when the oil content is excessively high (Manzoor et al., 2022).

Stability Observations

Emulsion stability was evaluated over a 14-day period. Initial stability was observed in CWBs containing a low oleogel content (5% w/w), which maintained a thick and creamy consistency due to the stabilizing effect of okra mucilage. However, at higher oleogel concentrations (10% w/w), emulsions began to lose stability, with visible phase separation observed by day 14.

Initial Stability (72 Hours)

Formulations with 5% w/w oleogel remained stable and retained a visually thick consistency, consistent with other studies showing that lower amounts of gelators tend to produce more stable emulsions (Sagiri & Poverenov, 2024). These emulsions were positioned near the boundary between Regions 1 and 2, where the optimal balance between oleogel and polysaccharide supports stable network formation.

Instability at 14 Days

Formulations with higher oleogel content (CWB2 and CWB4) exhibited phase separation after 14 days. The breakdown of the emulsion likely due to droplet coalescence and the insufficient stabilizing capacity of the polysaccharide during long-term storage resulted in a thick, fluffy layer at the bottom and a serum layer at the top. Similar phase separation behavior has been documented in studies on high-fat emulsions, where excess fat leads to destabilization over time (Bai et al., 2017; Bai et al., 2018; Dantas et al., 2021; Du Le et al., 2020; McClements et al., 2017).

Comparison to Alternative Stabilization Approaches

Although the discussion acknowledged the potential for exploring alternative stabilizers or combinations of stabilizers, referencing additional studies would provide a broader perspective. For instance, research has investigated the use of protein-based stabilizers such as whey protein isolate and sodium caseinate in the stabilization of high fat emulsions.

Additionally, the application of nanoparticle stablized emulsions has been proposed as an alternative to traditional emulsifier based systems (Binks, 2002; Bai et al., 2017; McClements et al., 2017; Dantas et al., 2021; Liang et al., 2023; Weerapol et al., 2024). Comparing the performance of the oleogel polysaccharide approach with these alternative strategies could further contextualize the advantages and limitations of the current CWB formulations.

Sensory and consumer acceptance considerations

The discussion primarily addressed the physicochemical properties and stability of the CWB formulations. However, incorporating references that explore sensory attributes, consumer acceptance, and potential health benefits of oleogel-based CWBs could enhance the relevance and applicability of the findings. Previous studies have examined the impact of oleogel-based emulsions on sensory properties and consumer preference in food products (Patel & Dewettinck 2015; Martins et al., 2018). Additionally, research has highlighted the health-promoting potential of oleogels, particularly in reducing saturated fat intake (Manzoor et al., 2022). Addressing these aspects would offer a more comprehensive understanding of the formulation's performance and its prospects for commercial success.

Implications for cocoa whey beverage formulation

The phase diagram analysis and stability assessments indicate that achieving long-term stability in CWBs requires precise control of both oleogel and stabilizer concentrations. The pseudo-ternary phase diagram serves as a valuable tool for identifying the optimal formulation window that ensures not only physical stability but also desirable texture and sensory appeal.

Optimal formulations

Formulations within Region 1 of the phase diagram demonstrated the best combination of stability and texture, making them particularly suitable for consumer oriented products that prioritize creaminess and extended shelf life. These formulations may benefit from further optimization, particularly in the selection and combination of polysaccharides. Exploring synergistic effects such as those between okra mucilage and other stabilizers could further enhance

long-term emulsion stability.

Microbiological Analysis of Cocoa Whey Beverages During Cold Storage

The microbiological quality of the beverage formulations was monitored during 14 days of cold storage at 5°C, as shown in Figure 5. Initially, the total plate counts were low, ranging from 2.26±0.04 to 2.63 ±0.09 log cfu/mL. After 14 days, these values increased slightly to a range of 3.12±0.12 to 3.30±0.10 log cfu/mL. Throughout the storage period, no significant differences in total bacterial counts were observed among the different treatments. Yeast and mold counts ranged from 0.40±0.05 to 0.59±0.01 log cfu/mL initially, increasing to between 1.12±0.02 and 1.39±0.07 log cfu/mL by the end of the storage period. The reduction in microbial growth observed across all treatments can likely be attributed to the efficiency of the heat treatment applied during processing, as well as the high sugar content of the beverages, which acts as a natural preservative. These findings are consistent with those reported by Nedanovska et al. (2022). Coliforms were not detected in any of the samples, suggesting that the thermal treatment was effective in eliminating potential contamination.

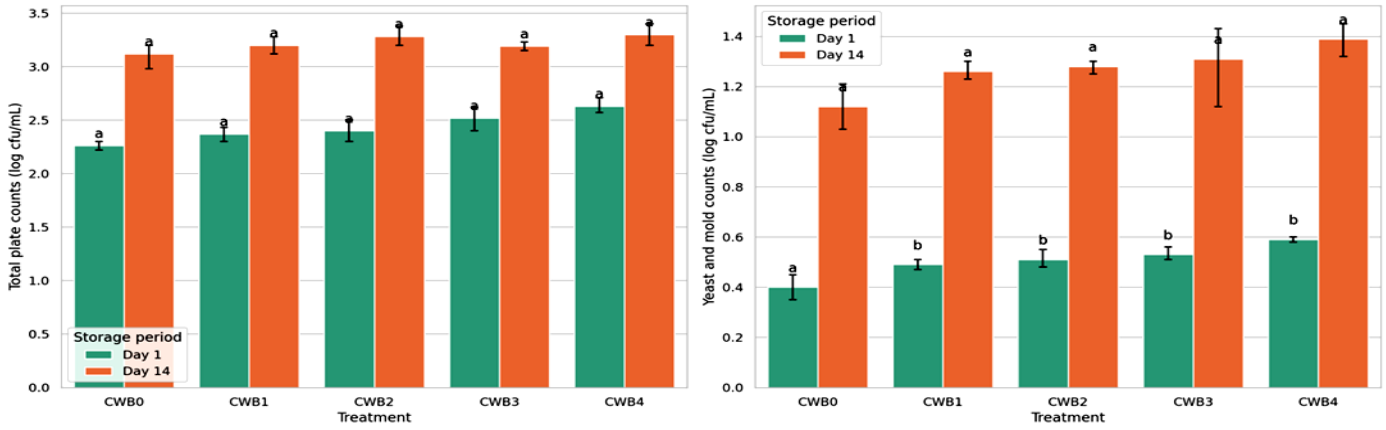

Figure 5. Changes in viable bacterial and yeast and mold counts (log cfu/mL) of cacao whey beverage treatments during the cold storage period

CWB0: Adjusted whey without adding oleogel and okra mucilage; **CWB1:** Formulation with 5% oleogel and 0.2% mucilage; **CWB2:** Formulation with 10% oleogel and 0.2% mucilage; **CWB3:** Formulation with 5% oleogel and 0.3% mucilage; **CWB4:** Formulation with 10% oleogel and 0.3% mucilage.

Future Research

Future studies should investigate the use of alternative stabilizers or combinations of polysaccharides to enhance the stability of formulations with higher oleogel content. Additionally, optimizing processing parameters such as homogenization pressure and temperature may further improve the microstructure and emulsifying properties of CWBs (Timms, 1984; Sagiri & Poverenov, 2024). These approaches could contribute to developing formulations with enhanced long-term stability and functional properties.

4. Conclusion

The incorporation of oleogel, prepared using a combination of beeswax, flaxseed oil, olive oil, and emulsifiers, along with okra polysaccharide, had a significant impact on the rheological and microstructural properties of the CWB formulations. Notably, the inclusion of oleogel effectively reduced the omega-6 to omega-3 fatty acid ratio, thereby improving the nutritional profile of the beverages. Formulations containing oleogel and a protein polysaccharide complex exhibited a marked increase in viscosity compared to the control. Furthermore, CWBs formulated with 5% oleogel and 0.3% mucilage demonstrated enhanced emulsion stability and uniformity, with a finely distributed microstructure. Overall, the integration of oleogel-structured emulsions and protein–mucilage polysaccharide complexes presents a promising strategy for improving the nutritional quality, stability, and sensory characteristics of cocoa whey-based beverages. These findings highlight the potential for developing innovative and functional dairy-based products using such formulation approaches.

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