2026 Volume: 02 Issue: 06 www.ijrpa.com ISSN 2456-9995 Review Article

International Journal Research Publication Analysis

Page: 01-22

ULTRASOUND-ASSISTED STABILIZATION OF CMC-BASED VIRGIN COCONUT OIL EMULSIONS FOR LOW-FAT SPREAD APPLICATIONS

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Article Received: 6 May 2026
Article Revised: 26 May 2026
Published on: 16 June 2026

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DOI: https://doi-doi.org/101555/ijrpa.8104

ABSTRACT

Virgin coconut oil (VCO) is a plant-based lipid phase with potential application in low-fat spread formulation. Still, its incorporation into aqueous food matrices requires a stable oil-in-water structure. This study evaluated the effects of Tween 80 and VCO concentrations on the physicochemical stability of ultrasonicated carboxymethyl cellulose (CMC)-stabilized VCO emulsions. Emulsions were prepared using VCO at 5, 10, and 15%, Tween 80 at 0, 0.5, 1.0, and 1.5%, fixed CMC at 0.5%, and distilled water as the continuous phase. Pre-emulsions were produced by magnetic stirring at 1000 rpm for 10 min, then treated with probe sonication at 50% amplitude for 5 min in pulse mode. Stability was characterized using the Turbiscan Stability Index, mean droplet diameter, zeta potential, viscosity, pH, peroxide value, and acid value. Tween 80 significantly reduced physical destabilization and droplet size, while VCO concentration significantly affected all measured parameters. Significant Tween 80 × VCO interactions were observed for TSI, droplet diameter, viscosity, and pH. The formulation containing 10% VCO, 1.0% Tween 80, 0.5% CMC, and 88.5% water showed the most balanced profile, with low TSI, small droplets, favorable zeta potential, suitable viscosity, and controlled lipid-quality indicators. This formulation is proposed as a promising basis for developing plant-based low-fat spreads.

KEYWORDS: Virgin coconut oil; Tween 80; carboxymethyl cellulose; ultrasonication; low-fat spread.

1. INTRODUCTION

Plant-based and reduced-fat food products have become increasingly important in food formulation because consumers and manufacturers seek products that combine health-oriented composition, desirable texture, and adequate shelf stability. Low-fat spreads are a particularly challenging product category because fat is not only a nutrient source but also a structural component that contributes to body, lubrication, mouthfeel, and resistance to phase separation. Therefore, reducing lipid content without compromising the semi-solid character of a spread requires a formulation strategy that organizes oil, water, stabilizers, and emulsifiers into a physically stable structure. Recent literature on plant-based double emulsions and emulsion gels shows that emulsion-based structuring can support the development of low-fat foods by reducing the total lipid fraction while preserving sensory and functional properties (Huang et al., 2025; Yiu et al., 2023; Abdullah et al., 2022).

Oil-in-water emulsions provide a suitable platform for low-fat spread bases because a dispersed lipid phase can be distributed within a continuous aqueous phase, while hydrocolloids and surfactants are used to build viscosity and inhibit destabilization. However, emulsions are thermodynamically unstable and may deteriorate through flocculation, coalescence, Ostwald ripening, creaming, sedimentation, or phase inversion during storage (Niu et al., 2023). These changes are especially relevant in low-fat spreads because even limited destabilization can alter appearance, texture, water release, spreadability, and consumer acceptability. Consequently, the development of a plant-based spread base requires integrated control of droplet formation, interfacial stabilization, continuous-phase viscosity, and chemical stability.

Virgin coconut oil is an attractive plant-based lipid phase because it is widely used in food applications and has a distinct functional identity. Nevertheless, direct incorporation of VCO into aqueous matrices is difficult because the oil and water phases are immiscible. Without adequate emulsification and interfacial stabilization, VCO droplets may rapidly aggregate, cream, or coalesce. Earlier studies on coconut oil and VCO emulsions indicate that surfactant selection, emulsifier concentration, dispersed oil fraction, and mechanical processing conditions strongly influence droplet size, stability, and physicochemical performance (Ramisetty et al., 2015; Li & Xiang, 2019; Miksusanti et al., 2023; Widianingrum et al., 2023).

Tween 80 is a non-ionic surfactant commonly used in oil-in-water systems because it reduces oil-water interfacial tension and promotes the formation of smaller droplets. Its high hydrophilic-lipophilic balance supports the stabilization of oil droplets in aqueous media, while its steric effect can help protect against aggregation. Surfactant concentration, however, must be optimized because insufficient levels cannot cover newly formed interfacial area, whereas excessive surfactant may not provide proportionate benefits and may be undesirable from formulation and labeling perspectives. HLB-based emulsion studies show that emulsion stability improves when the emulsifier system is compatible with the oil phase, supporting the need for concentration-specific optimization rather than simple surfactant maximization (Wang et al., 2023).

Ultrasonic emulsification provides an efficient mechanical route to reduce oil droplet size via acoustic cavitation. During sonication, the collapse of cavitation bubbles generates intense localized shear that disrupts the dispersed phase and supports finer droplet formation. Reviews of ultrasonic emulsification describe this approach as useful for producing nano- and fine-emulsions because it can intensify emulsification and improve control over droplet characteristics (Yao et al., 2022). Comparative work also shows that ultrasound-assisted emulsification can produce smaller droplets and better stability than conventional high-speed homogenization in selected oil-in-water systems (Zhou et al., 2021). These findings provide a strong basis for the use of probe sonication in VCO emulsion preparation.

In addition to the emulsifier and mechanical process, the aqueous-phase stabilizer is central to spread-base design. Carboxymethyl cellulose is a food-grade hydrocolloid used as a thickener, stabilizer, rheology modifier, water-retention agent, and texture improver (Costa et al., 2023). CMC can increase continuous-phase viscosity, restrict droplet mobility, and slow creaming. In protein-stabilized rice bran oil emulsions, CMC improved droplet charge, microstructure, viscosity, and storage stability, indicating its relevance as a continuous-phase stabilizing polymer (Zhang et al., 2022). However, CMC alone may not provide sufficient interfacial stabilization for oil droplets, so using it with a surfactant such as Tween 80 may provide more effective combined stabilization.

Emulsion stability cannot be reliably evaluated by visual observation alone, as early destabilization may occur before obvious phase separation is visible. A robust assessment should combine droplet size, zeta potential, viscosity, pH, lipid-quality parameters, and quantitative physical stability indicators. Turbiscan Stability Index is especially useful because it summarizes changes detected by static multiple light scattering along the height of the sample. In the present formulation context, TSI can be interpreted together with droplet diameter, zeta potential, viscosity, peroxide value, and acid value to determine whether a formulation is physically stable, chemically acceptable, and functionally relevant for low-fat spread development (Niu et al., 2023; ten Klooster et al., 2024).

Although VCO emulsions, Tween 80-based nanoemulsions, CMC-stabilized systems, and ultrasound-assisted emulsification have each been investigated, limited attention has been given to the combined effects of Tween 80 and VCO concentrations in ultrasonicated CMC-stabilized VCO emulsions intended as plant-based low-fat spread bases. Therefore, this study aimed to evaluate the physicochemical stability of such emulsions using a factorial formulation approach. It was hypothesized that Tween 80 and VCO concentration would significantly affect TSI, droplet size, zeta potential, viscosity, pH, peroxide value, and acid value, and that an intermediate VCO concentration with adequate Tween 80 would provide the most balanced formulation profile.

2. MATERIAL AND METHOD

2.1 Materials

Commercial food-grade virgin coconut oil from a single producer was used as the lipid phase. Food-grade Tween 80 was used as the emulsifier, food-grade carboxymethyl cellulose as the stabilizer and thickening agent, and distilled water as the continuous aqueous phase. Before emulsion preparation, pure VCO was analyzed for peroxide value and acid value to determine the initial chemical quality of the lipid phase. These indices are commonly used to evaluate oxidative and hydrolytic deterioration in coconut oil and other edible oils (Rangana & Wickramasinghe, 2023).

All ingredients were weighed on a 100 g formulation basis. The CMC concentration was fixed at 0.5% across all formulations to isolate the effects of Tween 80 and VCO concentrations. This design reflected the role of CMC as a constant, continuous-phase structuring agent, while Tween 80 and oil concentration were treated as the main formulation variables.

2.2 Experimental Design and Formulation

The study used a completely randomized factorial design with two factors: Tween 80 concentration and VCO concentration. Tween 80 was used at four levels: 0%, 0.5%, 1.0%, and 1.5%. The 0% Tween 80 treatment served as the control formulation to assess the stabilizing contribution of CMC in the absence of a low-molecular-weight surfactant. VCO was used at three levels: 5%, 10%, and 15%. Each formulation was prepared in three independent replications, resulting in 12 formulations and 36 independent emulsion batches. Factorial design is suitable for emulsion optimization because it enables the main and interaction effects of formulation components to be assessed statistically (Miksusanti et al., 2023).

All formulations were prepared on a weight/weight basis. Distilled water was adjusted to match the VCO and Tween 80 levels, whereas CMC remained fixed at 0.5%. The detailed formulation matrix is shown in Table 1.

Table 1: Formulation of CMC-stabilized VCO emulsions.

Formulation	VCO (%)	Tween 80 (%)	CMC (%)	Distilled water (%)
F1	5	0	0.5	94.5
F2	10	0	0.5	89.5
F3	15	0	0.5	84.5
F4	5	0.5	0.5	94.0
F5	10	0.5	0.5	89.0
F6	15	0.5	0.5	84.0
F7	5	1.0	0.5	93.5
F8	10	1.0	0.5	88.5
F9	15	1.0	0.5	83.5
F10	5	1.5	0.5	93.0
F11	10	1.5	0.5	88.0
F12	15	1.5	0.5	83.0

2.3 Preparation of VCO Emulsions

CMC was first dispersed in distilled water and hydrated until a homogeneous aqueous phase was obtained. Tween 80 was then added according to the formulation and mixed into the hydrated CMC solution. VCO was gradually introduced into the aqueous phase while stirring at 1000 rpm for 10 min with a magnetic stirrer to form a coarse pre-emulsion. Controlled pre-emulsification was used to distribute the oil phase before high-energy droplet disruption.

The pre-emulsion was processed using a probe sonicator at 50% amplitude for 5 min under pulse mode with 5 s on and 5 s off cycles. The sample was held in an ice bath during sonication to minimize the temperature rise induced by acoustic energy input. Probe sonication was selected because ultrasonic cavitation can disrupt oil droplets and support the formation of finer emulsions, as described in ultrasound-assisted emulsification studies (Yao et al., 2022; Zhou et al., 2021). All emulsions were prepared at room temperature without pH adjustment.

2.4 Physical and Physicochemical Characterization

Physical stability was evaluated using a Turbiscan or particle stability analyzer for 24 h at room temperature. Fresh emulsions were transferred into Turbiscan cells without dilution and scanned at predetermined intervals. The main response was the Turbiscan Stability Index after 24 h, while backscattering and transmission profiles were used to support the interpretation of droplet migration and destabilization. Multiple light scattering is appropriate for evaluating emulsion stability because it can detect early droplet aggregation and migration before macroscopic separation becomes visible (Niu et al., 2023; Zhou et al., 2021).

Mean droplet diameter was measured using a particle size analyzer. Samples were diluted with distilled water before measurement to avoid excessive turbidity and multiple scattering. Results were expressed in micrometers. Droplet size was included because it is directly related to emulsion stability, interfacial area, and creaming tendency (Niu et al., 2023; ten Klooster et al., 2024).

Zeta potential was measured using a zeta potential analyzer after suitable dilution with distilled water, and results were expressed in millivolts. Although Tween 80 is a non-ionic surfactant and can provide steric stabilization, zeta potential was measured to evaluate the net surface charge of droplets and the tendency toward electrostatic aggregation. Viscosity was measured using a Brookfield viscometer at 60 rpm and room temperature, and results were expressed in cP. pH was measured directly without dilution using a calibrated pH meter.

Peroxide value and acid value were determined directly from emulsion samples using standard AOCS/AOAC titration methods with appropriate adjustment for emulsion systems.

Peroxide value was expressed as meq O_2/kg emulsion, whereas acid value was expressed as mg KOH/g emulsion. These chemical indices were used to assess changes in primary oxidation and hydrolytic quality in the lipid-containing emulsion matrix.

2.5 Statistical Analysis

Data were expressed as mean ± standard deviation from three independent replications. A two-way analysis of variance was used to evaluate the effects of Tween 80 concentration, VCO concentration, and their interaction. When significant differences were observed, Tukey's test was used to compare means at $p < 0.05$. The statistical outputs were interpreted alongside practical formulation criteria for the development of a low-fat spread.

3. RESULT

3.1 Statistical Analysis Overview

Two-way ANOVA was conducted to determine whether Tween 80 concentration, VCO concentration, and their interaction affected the physicochemical characteristics of ultrasonicated CMC-stabilized VCO emulsions. The statistical summary is presented in Table 2. Tween 80 concentration significantly affected TSI, droplet diameter, zeta potential, viscosity, pH, and peroxide value. VCO concentration significantly affected all measured parameters. Significant Tween 80 × VCO interactions were observed for TSI, droplet diameter, viscosity, and pH, indicating that the effect of Tween 80 depended partly on the amount of dispersed oil.

Interaction effects were not significant for zeta potential, peroxide value, and acid value. Acid value was significantly affected only by VCO concentration, suggesting that the lipid phase was the dominant determinant of the hydrolytic quality response. This result supports the use of integrated formulation interpretation, because physical and chemical responses were not controlled by a single formulation variable.

Table 2: Two-way ANOVA summary for physicochemical properties of VCO emulsions.

Parameter	Tween 80 F	Tween 80 p	VCO F	VCO p	Interaction F	Interaction p
TSI	525.19	<0.001	113.43	<0.001	16.33	<0.001
Droplet diameter	1105.75	<0.001	91.42	<0.001	26.09	<0.001

Parameter	Tween 80 F	Tween 80 p	VCO F	VCO p	Interaction F	Interaction p
Zeta potential	230.51	<0.001	50.48	<0.001	1.12	0.380
Viscosity	138.00	<0.001	512.58	<0.001	10.41	<0.001
pH	25.45	<0.001	29.03	<0.001	4.23	0.005
Peroxide value	9.99	<0.001	722.11	<0.001	2.29	0.069
Acid value	0.47	0.708	209.32	<0.001	1.55	0.206

3.2 Physical Stability Based on Turbiscan Analysis

Physical stability was evaluated using TSI after 24 h of observation. Lower TSI values indicate smaller changes in the emulsion's optical profile and therefore greater physical stability. As shown in Table 3 and Figure 1, formulations without Tween 80 had the highest TSI values. In the absence of Tween 80, TSI increased from 16.24 ± 0.45 in F1 to 22.53 ± 2.50 in F2 and 30.34 ± 1.87 in F3. This trend shows that increasing the oil fraction, without a dedicated interfacial emulsifier, led to greater destabilization.

Adding Tween 80 substantially reduced TSI at all oil levels. At 0.5% Tween 80, TSI decreased to 7.78 ± 0.48, 11.53 ± 1.22, and 15.69 ± 1.50 for the 5, 10, and 15% VCO formulations, respectively. Further improvement was observed at 1.0 and 1.5% Tween 80. The lowest value was obtained in F10 with a TSI of 3.23 ± 0.29, although this formulation contained only 5% VCO. Among formulations with 10% VCO, F8 and F11 showed low TSI values of 4.31 ± 0.24 and 3.97 ± 0.39, respectively. These results agree with ultrasound and surfactant-based emulsion studies showing that adequate interfacial stabilization and droplet disruption improve storage stability (Zhou et al., 2021; Wang et al., 2023).

Table 3: Turbiscan Stability Index of CMC-stabilized VCO emulsions after 24 h observation.

Formulation	Tween 80 (%)	VCO (%)	TSI 24 h
F1	0	5	16.24 ± 0.45
F2	0	10	22.53 ± 2.50
F3	0	15	30.34 ± 1.87
F4	0.5	5	7.78 ± 0.48
F5	0.5	10	11.53 ± 1.22
F6	0.5	15	15.69 ± 1.50
F7	1.0	5	3.52 ± 0.84

Formulation	Tween 80 (%)	VCO (%)	TSI 24 h
F8	1.0	10	4.31 ± 0.24
F9	1.0	15	6.79 ± 0.35
F10	1.5	5	3.23 ± 0.29
F11	1.5	10	3.97 ± 0.39
F12	1.5	15	5.83 ± 0.75

Values are expressed as mean ± standard deviation (n = 3).

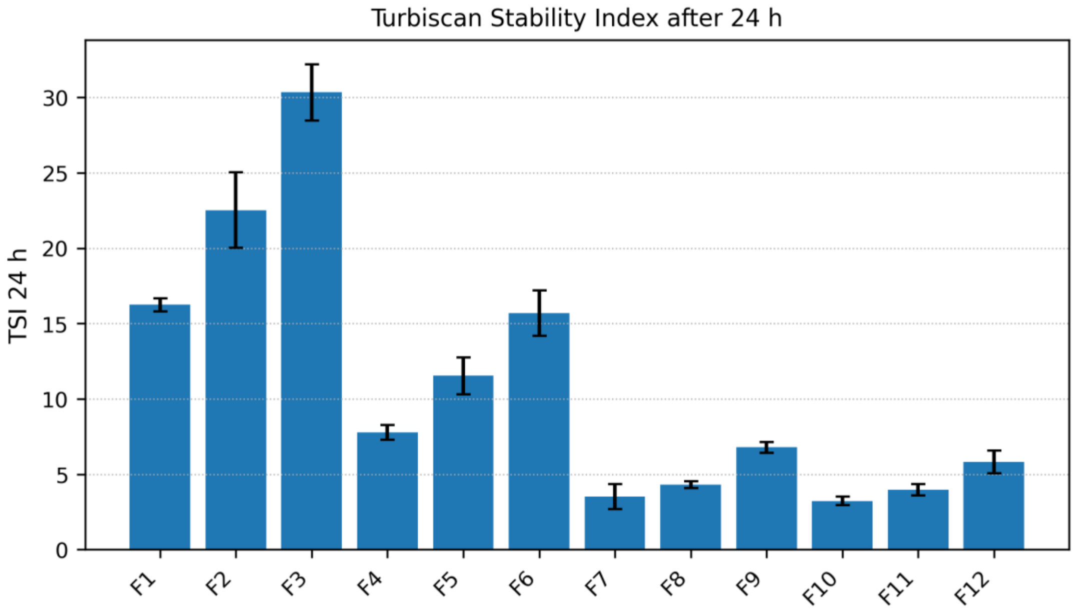

Figure 1: Turbiscan Stability Index of CMC-stabilized VCO emulsions during 24 h observation.

3.3 Mean Droplet Diameter

Mean droplet diameter is a major indicator of emulsion formation efficiency and stability. As shown in Table 4 and Figure 2, the control formulations produced the largest droplets, ranging from 9.42 ± 0.39 to 16.04 ± 1.21 µm. Droplet diameter increased with VCO concentration, particularly in the absence of Tween 80. This indicates that a higher oil fraction increased the demand for interfacial coverage during sonication.

The addition of Tween 80 sharply reduced droplet size. At 0.5% Tween 80, mean droplet diameter decreased to 3.72-5.85 µm. At 1.0 and 1.5% Tween 80, the droplets were further reduced to 0.87-2.14 µm. The smallest droplet size was observed in F10, which had 0.87 ± 0.04 µm. However, the formulation contained only 5% VCO and may not provide sufficient lipid body for a spread base. F8, containing 10% VCO and 1.0% Tween 80, produced a small droplet diameter of 1.53 ± 0.02 µm, making it more relevant for low-fat spread formulation. The droplet-size response is consistent with ultrasonic emulsification theory, where acoustic cavitation disrupts oil droplets and a suitable surfactant concentration stabilizes the newly formed interfacial area (Yao et al., 2022; Li & Xiang, 2019).

Table 4: Mean droplet diameter of ultrasonicated CMC-stabilized VCO emulsions.

Formulation	Tween 80 (%)	VCO (%)	Mean droplet diameter (µm)
F1	0	5	9.42 ± 0.39
F2	0	10	12.14 ± 0.91
F3	0	15	16.04 ± 1.21
F4	0.5	5	3.72 ± 0.36
F5	0.5	10	4.24 ± 0.15
F6	0.5	15	5.85 ± 0.22
F7	1.0	5	1.31 ± 0.06
F8	1.0	10	1.53 ± 0.02
F9	1.0	15	2.14 ± 0.09
F10	1.5	5	0.87 ± 0.04
F11	1.5	10	1.21 ± 0.09
F12	1.5	15	1.61 ± 0.11

Values are expressed as mean ± standard deviation (n = 3).

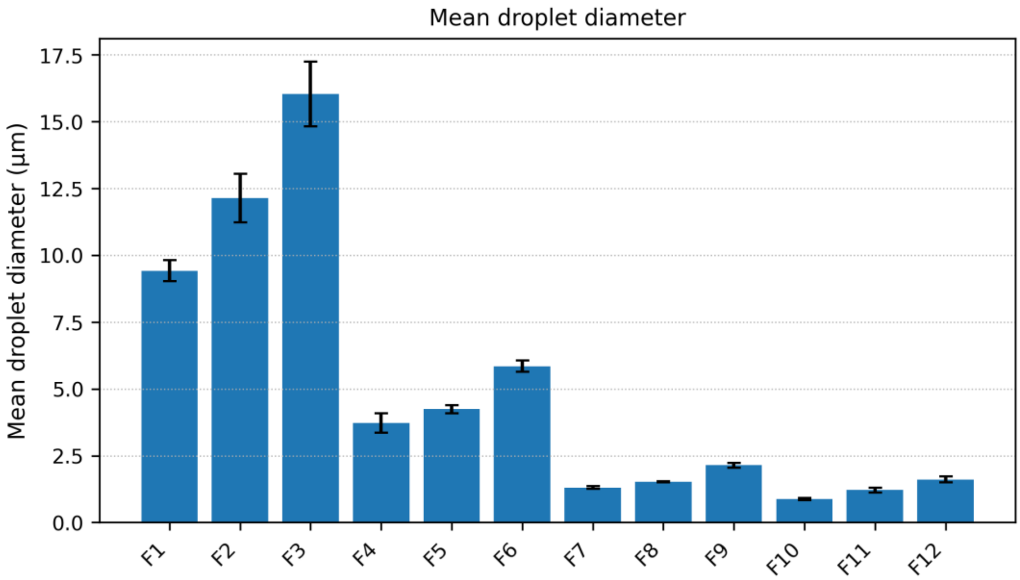

Figure 2: Mean droplet diameter of ultrasonicated CMC-stabilized VCO emulsions.

3.4 Zeta Potential

Zeta potential was used to evaluate droplet surface charge and aggregation tendency. Table 5 and Figure 3 show that the control formulations had zeta potential values closer to zero, ranging from -17.90 ± 0.22 to -11.34 ± 0.10 mV. These values indicate weaker electrostatic repulsion and are consistent with the high TSI and large droplet sizes observed in the same formulations.

Tween 80-containing formulations showed more negative zeta potential values, especially at 1.0 and 1.5% Tween 80. F8 showed a zeta potential of -29.09 ± 1.71 mV, supporting improved dispersion stability. Although Tween 80 is non-ionic and steric stabilization is expected to play a major role, the observed negative potential may reflect the combined influence of CMC, interfacial organization, and droplet composition. The non-significant interaction effect indicates that Tween 80 and VCO independently affected zeta potential within the tested range.

Table 5: Zeta potential of CMC-stabilized VCO emulsions.

Formulation	Tween 80 (%)	VCO (%)	Zeta potential (mV)
F1	0	5	-17.90 ± 0.22
F2	0	10	-15.06 ± 1.21
F3	0	15	-11.34 ± 0.10
F4	0.5	5	-26.03 ± 1.47
F5	0.5	10	-23.29 ± 1.68
F6	0.5	15	-19.53 ± 1.81
F7	1.0	5	-31.29 ± 0.94
F8	1.0	10	-29.09 ± 1.71
F9	1.0	15	-26.58 ± 1.19
F10	1.5	5	-30.06 ± 1.18
F11	1.5	10	-29.91 ± 1.59
F12	1.5	15	-26.40 ± 1.40

Values are expressed as mean ± standard deviation (n = 3).

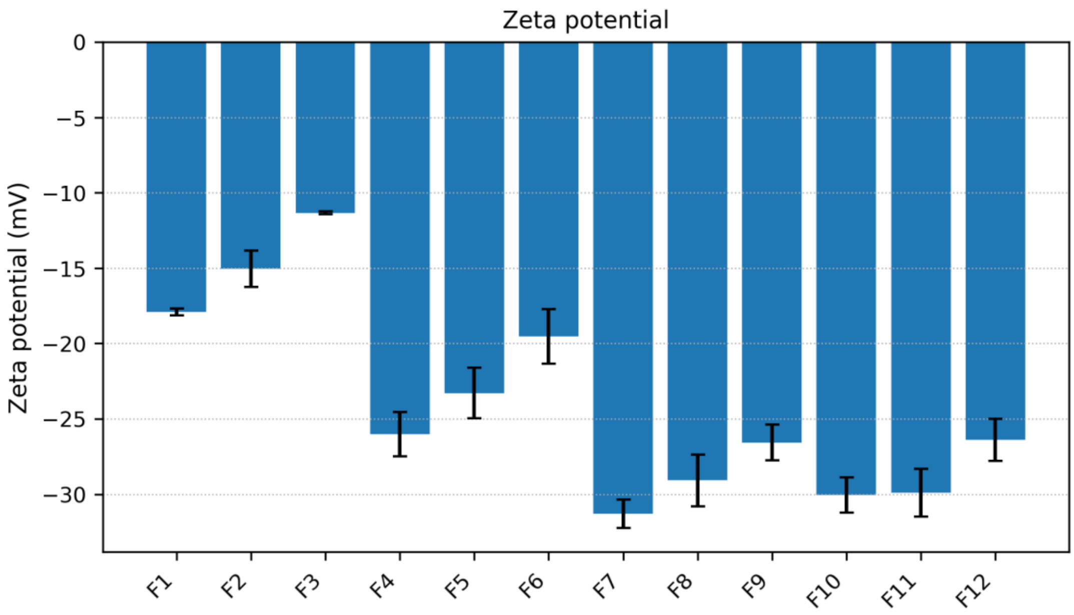

Figure 3: Zeta potential of CMC-stabilized VCO emulsions prepared with different Tween 80 and VCO concentrations.

3.5 Viscosity and pH

Viscosity is an essential property for a spread base because the product must have sufficient body and semi-thick consistency. Table 6 and Figure 4 show that viscosity increased with VCO concentration. The lowest viscosity was observed in F1 at 443.2 ± 16.7 cP, while the highest viscosity was observed in F12 at 1427.9 ± 42.3 cP. The increase in viscosity with increasing oil fraction indicates greater internal resistance to flow, likely due to a higher dispersed-phase volume and improved structuring within the CMC-containing continuous phase.

F8 and F11 showed balanced viscosity values of 972.7 ± 35.0 and 989.9 ± 40.0 cP, respectively. These values were more suitable for spread-based development than formulations with very low viscosity or excessive thickness. The viscosity response is consistent with CMC's known role as a rheology modifier and thickening agent in food systems (Costa et al., 2023; Zhang et al., 2022).

The pH values ranged narrowly from 6.39 ± 0.04 to 6.59 ± 0.02, as shown in Table 6 and Figure 5. Although pH was statistically affected by formulation factors, the practical variation was relatively small. Thus, the major differences in stability were more closely associated with Tween 80 concentration, VCO concentration, droplet diameter, zeta potential, and viscosity than with pH alone.

Table 6: Viscosity and pH of CMC-stabilized VCO emulsions.

Formulation	Tween 80 (%)	VCO (%)	Viscosity (cP)	pH
F1	0	5	443.2 ± 16.7	6.59 ± 0.02
F2	0	10	622.8 ± 56.2	6.51 ± 0.03
F3	0	15	907.0 ± 29.7	6.56 ± 0.02
F4	0.5	5	504.4 ± 14.7	6.55 ± 0.01
F5	0.5	10	755.7 ± 23.5	6.52 ± 0.01
F6	0.5	15	954.7 ± 79.2	6.47 ± 0.03
F7	1.0	5	640.4 ± 72.7	6.51 ± 0.02
F8	1.0	10	972.7 ± 35.0	6.47 ± 0.01
F9	1.0	15	1324.6 ± 60.1	6.43 ± 0.03
F10	1.5	5	645.9 ± 6.8	6.52 ± 0.03
F11	1.5	10	989.9 ± 40.0	6.47 ± 0.05
F12	1.5	15	1427.9 ± 42.3	6.39 ± 0.04

Values are expressed as mean ± standard deviation (n = 3).

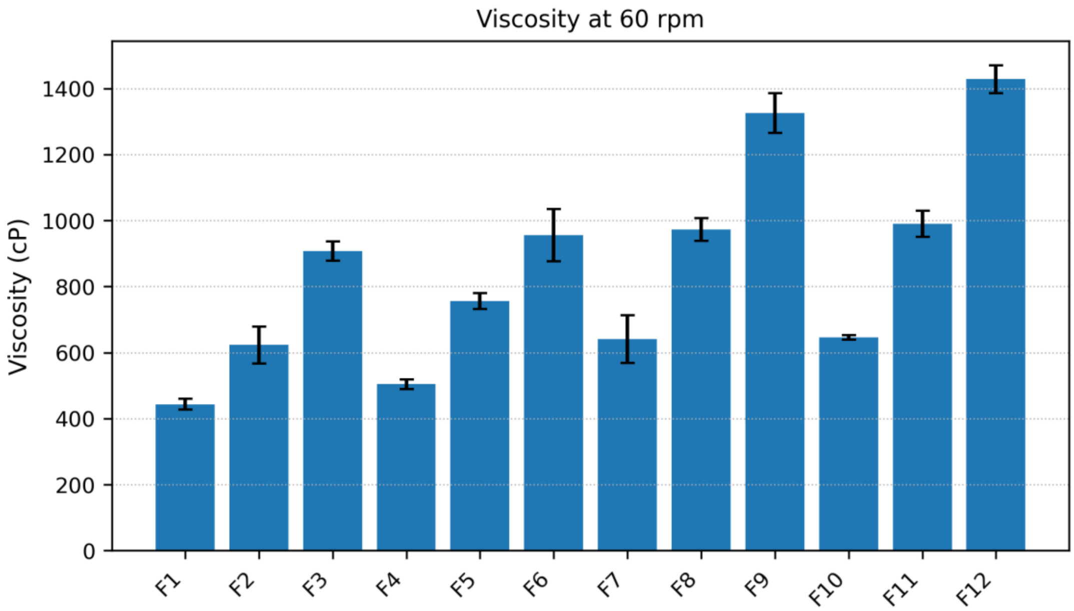

Figure 4: Viscosity of VCO emulsions measured using a Brookfield viscometer at 60 rpm.

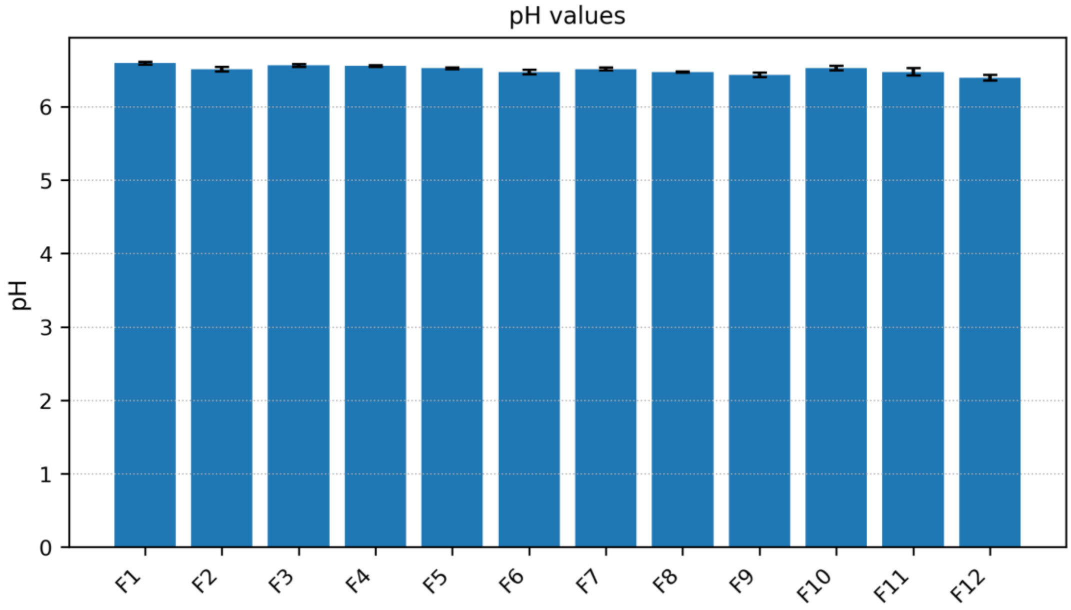

Figure 5: pH values of CMC-stabilized VCO emulsions.

3.6 Peroxide Value and Acid Value

Peroxide value and acid value were used to evaluate chemical stability. As shown in Table 7 and Figure 6, the peroxide value increased with increasing VCO concentration. F8 showed a peroxide value of 0.406 ± 0.032 meq O_2/kg emulsion, which was lower than the peroxide values of formulations containing 15% VCO. The results indicate that increasing the oil fraction increased the amount of oxidizable lipid material in the emulsion matrix.

Acid value also increased with VCO concentration, as shown in Figure 7. F8 showed an acid value of 0.0792 ± 0.0047 mg KOH/g emulsion, whereas F12 reached 0.1138 ± 0.0117 mg KOH/g emulsion. ANOVA confirmed that acid value was significantly affected by VCO concentration but not by Tween 80 or the Tween 80 × VCO interaction. This indicates that the hydrolytic quality response was governed mainly by the lipid phase. The chemical stability trends agree with the use of peroxide value and acid value as standard indicators of oxidative and hydrolytic quality in VCO and edible oil systems (Rangana & Wickramasinghe, 2023; ten Klooster et al., 2024).

Table 7: Peroxide value and acid value of CMC-stabilized VCO emulsions.

Formulation	Tween 80 (%)	VCO (%)	PV (meq O_2/kg)	Acid value (mg KOH/g)
F1	0	5	0.232 ± 0.027	0.0471 ± 0.0014
F2	0	10	0.486 ± 0.020	0.0750 ± 0.0054

Formulation	Tween 80 (%)	VCO (%)	PV (meq O_2/kg)	Acid value (mg KOH/g)
F3	0	15	0.772 ± 0.037	0.1049 ± 0.0050
F4	0.5	5	0.197 ± 0.018	0.0433 ± 0.0071
F5	0.5	10	0.463 ± 0.044	0.0767 ± 0.0061
F6	0.5	15	0.780 ± 0.022	0.1071 ± 0.0035
F7	1.0	5	0.205 ± 0.004	0.0383 ± 0.0104
F8	1.0	10	0.406 ± 0.032	0.0792 ± 0.0047
F9	1.0	15	0.652 ± 0.022	0.1008 ± 0.0118
F10	1.5	5	0.186 ± 0.021	0.0474 ± 0.0055
F11	1.5	10	0.436 ± 0.072	0.0690 ± 0.0091
F12	1.5	15	0.693 ± 0.034	0.1138 ± 0.0117

Values are expressed as mean ± standard deviation (n = 3).

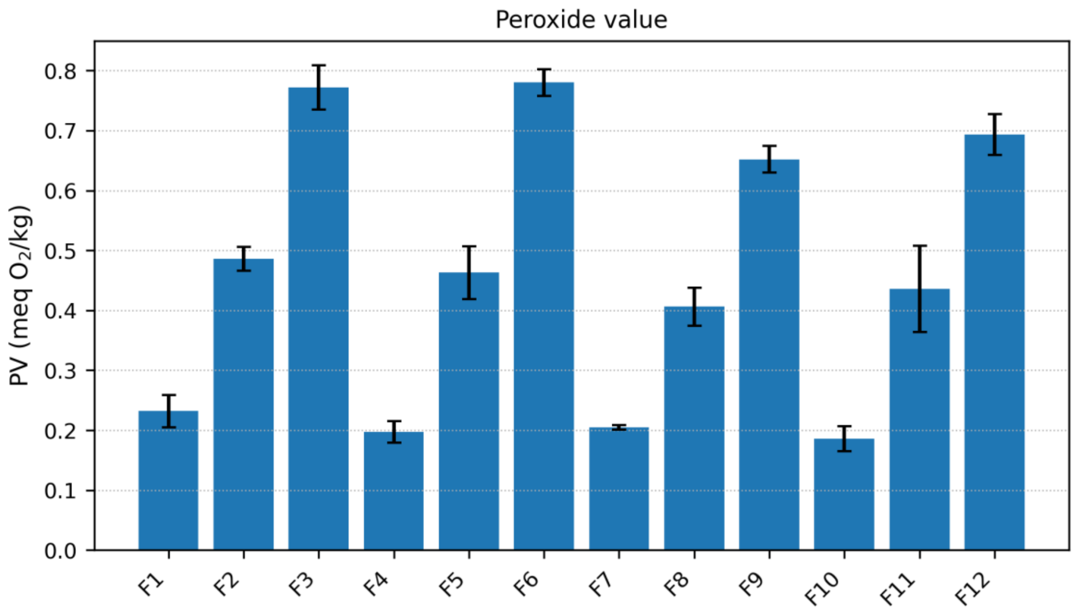

Figure 6: Peroxide value of VCO emulsions prepared with different Tween 80 and VCO concentrations.

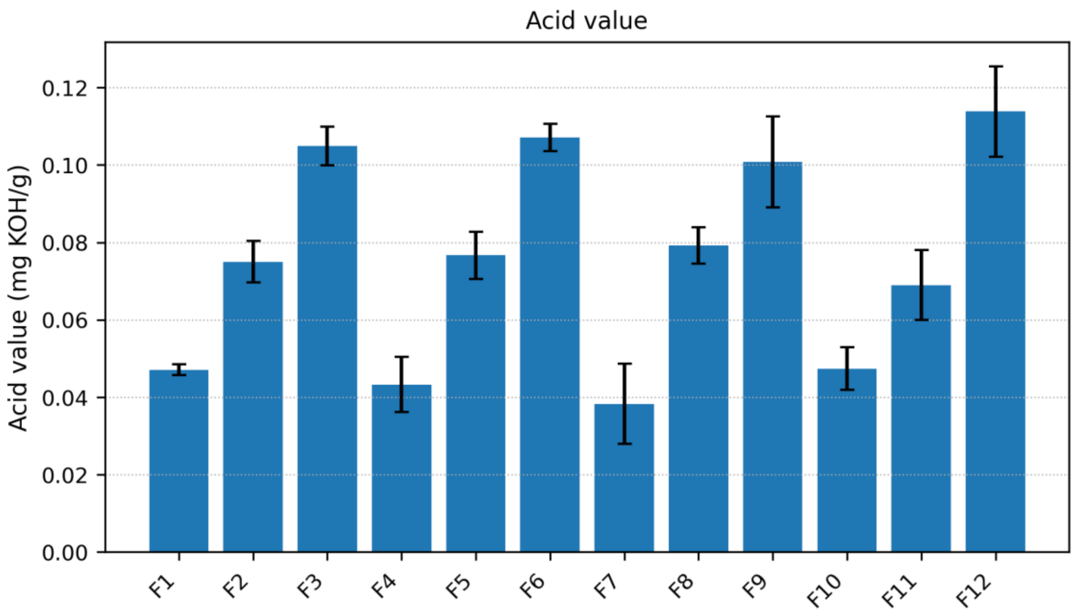

Figure 7: Acid value of VCO emulsions prepared with different Tween 80 and VCO concentrations.

3.7 Selection of the Best Formulation

The best formulation was selected using integrated interpretation rather than a single response value. Table 8 compares the formulations with the most favorable physical stability profiles, particularly those containing 1.0 and 1.5% Tween 80. F10 showed the lowest TSI and smallest droplet size, with a TSI of 3.23 and a droplet diameter of 0.87 µm. However, this formulation contained only 5% VCO, which may be insufficient to provide lipid body and mouthfeel in a spread-type product.

F11 also showed good stability and a balanced viscosity, but it required 1.5% Tween 80. F8 was selected as the most promising formulation because it contained 10% VCO, 1.0% Tween 80, 0.5% CMC, and 88.5% distilled water while maintaining low TSI, small droplet size, favorable zeta potential, suitable viscosity, and controlled chemical quality indicators. This integrated selection approach is consistent with the development of low-fat emulsions, where physical stability, chemical quality, texture, and practical formulation relevance must be evaluated together (Huang et al., 2025; Yiu et al., 2023).

Table 8: Comparison of selected VCO emulsion formulations.

Formulation	Tween 80 (%)	VCO (%)	TSI 24 h	Droplet (µm)	Zeta (mV)	Viscosity (cP)	PV	Acid value
F7	1.0	5	3.52	1.31	-31.29	640.4	0.205	0.0383

Formulation	Tween 80 (%)	VCO (%)	TSI 24 h	Droplet (µm)	Zeta (mV)	Viscosity (cP)	PV	Acid value
F8	1.0	10	4.31	1.53	-29.09	972.7	0.406	0.0792
F9	1.0	15	6.79	2.14	-26.58	1324.6	0.652	0.1008
F10	1.5	5	3.23	0.87	-30.06	645.9	0.186	0.0474
F11	1.5	10	3.97	1.21	-29.91	989.9	0.436	0.0690
F12	1.5	15	5.83	1.61	-26.40	1427.9	0.693	0.1138

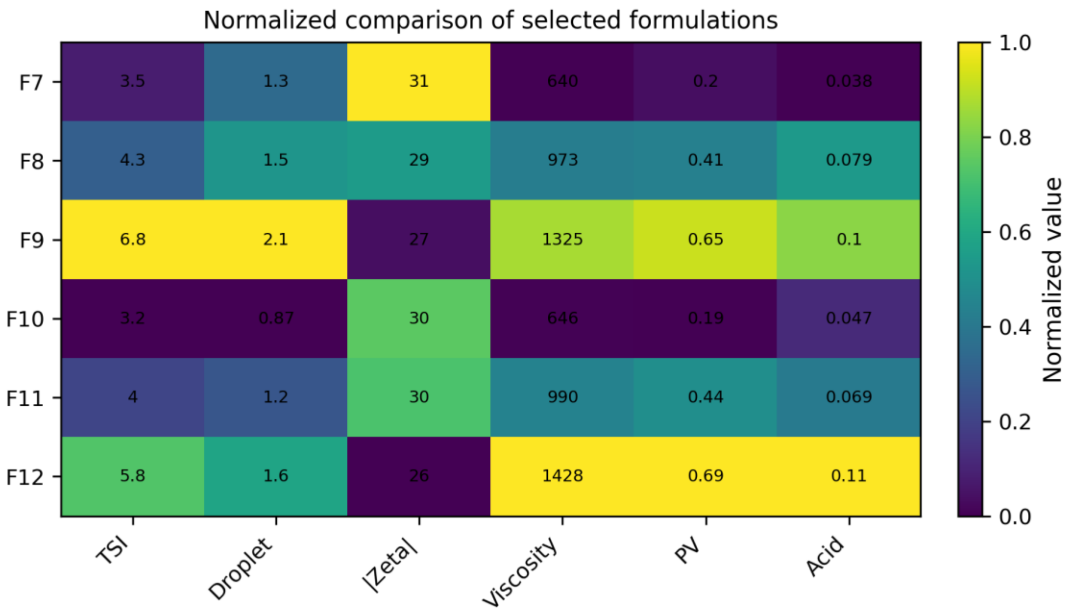

Figure 8: Overall comparison of selected VCO emulsion formulations for plant-based low-fat spread base.

4. DISCUSSION

The results demonstrate that Tween 80 was essential for the development of physically stable ultrasonicated CMC-stabilized VCO emulsions. In the absence of Tween 80, TSI values remained high, increasing from 16.24 ± 0.45 to 30.34 ± 1.87 as the VCO concentration increased from 5 to 15%. This pattern indicates that 0.5% CMC alone was insufficient to stabilize the oil-water interface when the dispersed lipid fraction increased. CMC can thicken the aqueous phase and reduce droplet mobility, but forming a stable oil-in-water emulsion also requires sufficient interfacial coverage. This interpretation aligns with CMC's role as a hydrocolloid stabilizer and rheology modifier rather than a complete substitute for an optimized surfactant system (Costa et al., 2023; Zhang et al., 2022).

The reduction in TSI and droplet diameter after the addition of Tween 80 reflects the combined effects of interfacial tension reduction and ultrasound-induced droplet disruption. Probe sonication provides cavitation forces that break the oil phase into smaller droplets, while Tween 80 stabilizes the newly generated interfacial area. Without enough surfactant, droplet recoalescence can occur after sonication because the newly formed interface remains insufficiently protected. This explains why the control formulations had larger droplets of 9.42-16.04 µm, whereas Tween-containing formulations at 1.0 and 1.5% had droplets of 0.87-2.14 µm. Similar relationships among ultrasound, surfactant loading, and droplet-size reduction have been reported in coconut oil and other oil-in-water emulsions (Ramisetty et al., 2015; Li & Xiang, 2019; Yao et al., 2022; Zhou et al., 2021).

The influence of VCO concentration was evident across all measured parameters. Increasing oil fraction increased droplet diameter, viscosity, peroxide value, and acid value. From a physical perspective, higher oil content increases the dispersed-phase volume and increases the demand for surfactant adsorption during emulsification. When surfactant coverage is insufficient or near its limit, droplets may become larger and more prone to migration or aggregation. From a rheological perspective, a higher oil fraction increases the internal resistance to flow, which explains why 15% VCO formulations such as F9 and F12 reached cP values of 1324.6 ± 60.1 and 1427.9 ± 42.3, respectively. Although these high viscosities may increase body, they may also produce excessive thickness for a spread base, particularly if spreadability and consumer handling are considered.

The zeta potential results further support the stability interpretation. Control emulsions had zeta potential values close to zero, ranging from -17.90 ± 0.22 to -11.34 ± 0.10 mV, indicating weaker repulsive forces and a greater tendency toward aggregation. Tween 80-containing emulsions showed more negative values, with F8 reaching -29.09 ± 1.71 mV. Although Tween 80 is non-ionic, steric stabilization and changes in interfacial composition may alter the apparent surface environment of the droplets. The improved zeta potential, smaller droplet size, and lower TSI in F8 indicate that physical stabilization was governed not by a single parameter but by a combination of interfacial protection, droplet-size reduction, polymer-assisted viscosity, and reduced droplet mobility.

The chemical stability results show that increasing VCO concentration increases the peroxide and acid values. This trend is expected because a higher lipid fraction provides more substrate for primary oxidation and hydrolytic quality changes. F8 had a peroxide value of 0.406 ± 0.032 meq O_2/kg emulsion and acid value of 0.0792 ± 0.0047 mg KOH/g emulsion, while 15% VCO formulations generally showed higher values. These data indicate that 10% VCO provided a more balanced chemical-quality profile than 15% VCO under the tested formulation conditions. However, droplet-size reduction may have complex implications for oxidation because smaller droplets increase the oil-water interfacial area. Therefore, future storage studies should examine whether the physically stable small-droplet systems maintain acceptable oxidative stability during longer storage (ten Klooster et al., 2024; Rangana & Wickramasinghe, 2023).

The selection of F8 illustrates the importance of integrated formulation optimization. F10 had the lowest TSI and smallest droplet size, but its 5% VCO level may be too low to provide adequate lipid body and mouthfeel for a low-fat spread. F11 had good stability and viscosity but required 1.5% Tween 80. In contrast, F8 achieved a balanced response using 10% VCO and 1.0% Tween 80. This formulation maintained low TSI, small droplet size, favorable zeta potential, intermediate viscosity, and controlled peroxide and acid values. For product development, such a balance is more important than optimizing a single physical indicator. This view is consistent with the literature on low-fat and plant-based emulsion gels, where physical stability, chemical stability, texture, sensory performance, and practical applicability must be considered together (Huang et al., 2025; Yiu et al., 2023; Wan et al., 2023).

The practical implication of the findings is that ultrasonicated CMC-stabilized VCO emulsions can be formulated as a structured, plant-based, low-fat spread base when surfactant and oil concentrations are controlled simultaneously. The results also show that CMC should be used as part of a combined stabilization strategy rather than as the sole stabilizing agent. The most effective system in this study was achieved by combining interfacial stabilization with Tween 80, mechanical droplet disruption via probe sonication, and aqueous-phase viscosity enhancement with CMC. This combination provides a rational formulation pathway for reducing lipid content while maintaining functional emulsion properties relevant to spread applications.

Despite these promising findings, further work is required before product application. The current formulation study focused on initial physicochemical stability and 24 h Turbiscan analysis. A complete low-fat spread development program should include storage stability under refrigerated and room-temperature conditions, texture profile analysis, spreadability, water release, sensory acceptance, microbiological safety, packaging effects, and regulatory suitability. Because VCO quality may vary with source, processing method, and initial chemical characteristics, future work should also compare different VCO sources and evaluate whether the selected formulation remains stable across variations in raw materials (Rangana & Wickramasinghe, 2023).

5. CONCLUSION

Tween 80 and VCO concentrations significantly influenced the physicochemical stability of ultrasonicated CMC-stabilized VCO emulsions. Formulations without Tween 80 showed poor stability, as reflected by high TSI values, large droplet diameters, and zeta potentials closer to zero. Increasing VCO concentration increased viscosity but also increased droplet diameter, peroxide value, and acid value, indicating that a higher oil fraction affected both physical stability and chemical quality. The addition of Tween 80 improved physical stability by reducing droplet size and lowering TSI, especially at 1.0 and 1.5% concentrations. Among the tested formulations, F8, containing 10% VCO, 1.0% Tween 80, 0.5% CMC, and 88.5% distilled water, provided the most balanced profile. This formulation combined low TSI, small droplet diameter, favorable zeta potential, suitable viscosity, and controlled peroxide and acid values. The study contributes formulation-level evidence for the development of plant-based low-fat spread bases using combined surfactant stabilization, hydrocolloid thickening, and ultrasonic emulsification. Further studies should evaluate storage stability, texture, spreadability, sensory acceptance, microbial safety, and regulatory suitability to confirm the practical application of this formulation in low-fat spread products.

Acknowledgement

The authors would like to acknowledge the administrative and technical support provided by the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Tadulako University. The authors also appreciate the material contributions and equipment support provided by the laboratory facilities.

Declaration of Conflicting Interests

The authors declare no potential conflicts of interest with respect to the research, authorship and publication of this article.

FUNDING

The author received no financial support for the research, authorship, and publication of this article.

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