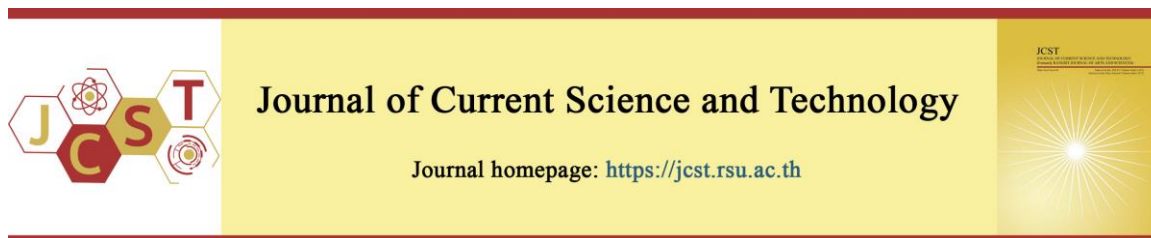


Cite this article: Hunsub, P., Khumpagpli, P., Srisomsak, S., Thephuttee, N., Apisittiwong, T., Pothinuch, P., Laksanalamai, V., & Prichapan, N. (2025). Production of reduced-fat plant-based salad dressing stabilized with carboxymethyl cellulose and aquafaba. *Journal of Current Science and Technology*, 15(4), Article 136. <https://doi.org/10.59796/jcst.V15N4.2025.136>



Production of Reduced-fat Plant-based Salad Dressing Stabilized with Carboxymethyl Cellulose and Aquafaba

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Received 11 March 2025; Revised 23 May 2025; Accepted 25 June 2025; Published online 20 September 2025

Abstract

In recent years, plant-based foods have gained substantial momentum around the world. Egg yolk, a key ingredient, is commonly used as an emulsifier in most salad dressings. Aquafaba was employed as an egg substitute in the development of plant-based salad dressings. Additionally, carboxymethyl cellulose (CMC) was incorporated as a stabilizing agent to enhance the emulsion stability of the formulation. The results showed that the control salad dressing (made with egg yolk) and the plant-based salad dressing containing 0.2–0.3 wt% CMC were highly stable with an emulsion stability index (ESI) of more than 90%, and a ζ -potential of approximately ± 30 mV. To develop a healthier, lower-calorie alternative, reduced-fat plant-based salad dressings were formulated by decreasing the oil content and substituting it with chickpea-derived aquafaba. The results demonstrated that the oil used in the formulation could be reduced from 60 wt% to 45 wt% without significantly affecting the ESI. The droplet diameter of the reduced-fat plant-based salad dressing containing 45 wt% oil was significantly smaller than that of the full-fat formulation with 60 wt% oil. Furthermore, *Wolffia* was incorporated into the formulation to develop a functional salad dressing with enhanced health benefits. Fortification with *Wolffia* significantly decreased the lightness, redness, ESI, and ζ -potential of the plant-based salad dressing. However, fortification with *Wolffia* did not significantly affect the yellowness of the plant-based salad dressing. The research findings could benefit further research efforts focused on the development of alternative health-promoting food products.

Keywords: carboxymethyl cellulose; chickpea; egg replacement; superfood; vegan salad dressing; *Wolffia globosa*

1. Introduction

Driven by customers who are concerned about their well-being and health problems, allergenic foods, environmental sustainability, and animal welfare, plant-based foods have gained significant momentum worldwide, including in Thailand, over the past few years. One of the ingredients in most salad dressings, which are an oil-in-water emulsion sold on the market today, is egg yolk, which contains a naturally occurring emulsifier that facilitates the dispersion of vegetable oil with water-based

ingredients, enhancing the stability of the emulsion. Egg allergies are also the most common food allergies caused by egg protein (Puangwerakul et al., 2022; Wongwailikhit et al., 2025). Finding a plant-based ingredient that can replace egg yolk has been a challenge for many researchers. Aquafaba, or pulse cooking water, which contains leached proteins from pulses, has been successfully used as an egg replacer with its interesting properties of being an emulsifier (Włodarczyk et al., 2022). Chickpea (*Cicer arietinum* L.) is a pulse and a rich source of high-quality plant-based

protein, making it an essential dietary ingredient for vegetarians and individuals who cannot digest or afford animal protein (Begum et al., 2023). Additionally, chickpeas contain bioactive compounds, including vitamins and antioxidants, which contribute to their health-promoting properties (Begum et al., 2023; Carbas et al., 2023). Previous studies showed that chickpea aquafaba is an effective egg replacer for producing a vegan full-fat mayonnaise (Włodarczyk et al., 2022). Wolffia, a small green aquatic plant, is consumed as food in several Asian countries, including Thailand. Wolffia has a good nutritional profile, with proteins, polysaccharides, vitamins, minerals, and other phytochemicals. Due to its high nutritional value as a healthier food alternative, it has been proposed as a plant-based ingredient for functional food products (Appenroth et al., 2018; Dhamaratana et al., 2025; Hu et al., 2022).

In this research, a fat-reduced plant-based salad dressing was developed. The replacement of egg yolk by a combination of chickpea aquafaba and a cellulose-derived stabilizing agent, CMC, was investigated. The appropriate amount of Wolffia powder was also studied in order to enhance the protein content of the product. Various quality aspects of the salad dressings, including ζ -potential, droplet diameter, emulsion stability index (ESI), microstructure, and colorimetric parameters and properties, were assessed to observe the effects of ingredient modifications. The obtained products could be an alternative for vegan, egg-allergic, and health-conscious consumers.

2. Objectives

To investigate the effects of CMC concentration, oil: aquafaba ratio, and Wolffia concentration on the qualities of reduced-fat plant-based salad dressing.

3. Materials and Methods

3.1 Materials

The ingredients used in this study consisted of dry chickpeas (Tawan Produce Co., Ltd., Samutprakarn, Thailand), refined rice bran oil containing 8,000 ppm oryzanol (Thai Edible Oil Co., Ltd., Bangkok, Thailand), distilled white vinegar containing 5% acetic acid (Kewpie (Thailand) Co., Ltd., Ratchaburi, Thailand), potable water (All Springs Co., Ltd., Pathum Thani, Thailand), granulated sugar (Mitr Phol Sugar Corp., Ltd., Suphanburi, Thailand), iodized table salt (Thai Refined Salt Co., Ltd., Bangkok, Thailand), yellow mustard composed of mustard seed, turmeric, spices, salt, distilled white vinegar, water, and natural flavor (Kraft Heinz Inc., Chicago, Illinois,

USA), egg yolk manually separated from whole eggs (CPF (Thailand) Co., Ltd., Bangkok, Thailand), CMC (Chemipan Co., Ltd., Bangkok, Thailand), and Wolffia powder (Vitoon Farm, Phitsanulok, Thailand).

3.2 Preparation of Chickpea Aquafaba

The method for the preparation of chickpea aquafaba was modified from He et al. (2021b). The chickpeas were cleaned and soaked in water at a ratio of 1:4 (chickpea: water), covered with cheesecloth, and then kept at 4°C for 16 hours (in a refrigerator). After soaking, the water was drained, and the soaked chickpeas were washed. The soaked chickpeas were then boiled for 2 hours at a ratio of 1:10 (soaked chickpea: boiling water). After boiling, the remaining water, referred to as aquafaba, was separated from the boiled chickpeas and then weighed. The aquafaba was reboiled to evaporate the excess water until the weight was equal to that of the soaked chickpeas. It was then cooled to 30°C, packed in a polyethylene bag, and stored at -20°C. The proximate composition of the prepared aquafaba was also analyzed using AOAC methods (AOAC International, 2023).

3.3 Effect of CMC Concentration on Qualities of Plant-based Salad Dressing

The control salad dressing (prepared with egg yolk) used in this research was modified from Manomaiwajee (2012). The formula ingredients are shown in Table 1, which included rice bran oil, distilled white vinegar, potable water, granulated sugar, iodized table salt, yellow mustard, and egg yolk. Except for rice bran oil, all other ingredients were weighed according to the formula and blended for 30 seconds in a 2 L blending container (SHARP EMC-15, Thai City Electric Co., Ltd., Bangkok, Thailand). Rice bran oil was gradually added to the mixture while the blender was running. Once all the oil had been added, blending continued for an additional 90 seconds. The obtained salad dressing was packed in a zip-lock bag and stored in a refrigerator at 4°C until further analysis.

The plant-based salad dressing samples were prepared using a formula similar to that of the control sample, with the exception that potable water and egg yolk were replaced with chickpea aquafaba (Table 1). The chickpea aquafaba functioned as an emulsifier, replacing the naturally occurring lecithin and protein found in egg yolk. Moreover, CMC (Chemipan Co., Ltd., Bangkok, Thailand) was incorporated as a stabilizer to enhance the emulsion stability of the

dressing. The concentrations of CMC in the samples were varied from 0–0.4 wt% (of the total amount of ingredient). To incorporate the CMC, it was first soaked in 24 g of chickpea aquafaba for 5 minutes, then mixed with the other ingredients according to the dressing preparation method described above. The qualities of all samples, including their zeta potential and emulsion stability index (ESI), were determined, and the appropriate amount of CMC was selected for use in the next experiment.

Table 1 Control and plant-based salad dressing formula

Ingredient list	Control formula		Plant-based formula	
	Weight (g)	% (w/w)	Weight (g)	% (w/w)
Rice bran oil	67	62.0	67	62.0
Distilled white vinegar	7	6.5	7	6.5
Potable water	12	11.1	0	0.0
Granulated sugar	7	6.5	7	6.5
Iodized table salt	1	0.9	1	0.9
Yellow mustard	2	1.9	2	1.9
Egg yolk	12	11.1	0	0.0
Chickpea aquafaba	0	0.0	24	22.2

Values represent mean of triplicates.

3.4 Effect of Oil: Aquafaba Ratio on Qualities of Plant-based Salad Dressing

Another set of plant-based salad dressing samples was prepared based on the formula and method from the previous experiment, but the amount of rice bran oil was reduced and replaced with an equal amount of chickpea aquafaba. The different weight ratios of rice bran oil to chickpea aquafaba were 60:20, 45:35, 30:50, and 15:65 (oil amounts: 50.6, 38.0, 25.3, and 12.7 wt%; aquafaba amounts: 16.9, 29.5, 42.1, and 54.8 wt%, respectively). The samples were then determined for quality characteristics, including their microstructure, droplet diameter, ESI, and viscosity. The optimal level of oil reduction was then selected for use in the next experiment.

3.5 Effect of Wolffia Concentration on Qualities of Plant-based Salad Dressing

The samples of Wolffia fortified salad dressing were prepared using the formula with suitable oil reduction obtained from the above experiment by the addition of Wolffia powder at different levels of 0, 0.15, 0.30, and 0.45 wt% (of the total amount of ingredients). The plant-based salad dressing samples

were prepared based on the formula and method achieved from the above experiment, except that the Wolffia powder was added before being mixed with oil. Qualities of the samples, including their instrumental color, zeta potential, and ESI, were determined, and the appropriate level of Wolffia in the salad dressing was selected. The finalized sample with the suitable amount of Wolffia was then analyzed for proximate composition.

3.6 Evaluation of Salad Dressing Qualities

3.6.1 Zeta Potential and Droplet Diameter

The sample was diluted with a 0.1 M phosphate buffer solution (pH 3.0) to an approximate concentration of 0.05% (w/w). The diluted sample was then transferred into a cuvette before measuring the zeta potential (ζ -potential) and droplet diameter using a dynamic light scattering (DLS) particle analyzer (Zetasizer Nano-ZS ZEN 3600, Malvern Panalytical Ltd., Malvern, UK).

3.6.2 Emulsion Stability Index (ESI)

The method used was modified from Mun et al. (2009). Fifteen g of sample was weighed (F_0) and transferred into a centrifuge tube with a screw cap, then centrifuged at 3,000 RPM for 15 minutes at 25°C. The cream layer on top was removed, and the remaining separated layer at the bottom was weighed (F_1). ESI expressed as a percentage was calculated as shown in Eq.1.

$$\text{ESI (\%)} = [(F_0 - F_1) / F_0] \times 100 \quad (1)$$

3.6.3 Microstructure

The 10x diluted sample (using water as diluent) was observed through a light microscope (Motic BA200, Motic Microscopes, Texas, USA). One drop of each sample was placed onto a 1 mm thick glass microscope slide (25×75 mm in width and length) using a plastic transfer pipette and covered with a cover slip. The illumination source in the microscope was a 20-watt quartz halogen lamp set to maximum intensity. A combination of objective and eyepiece at 40x and 10x magnification was used. Micrographs of the sample were obtained using a smartphone equipped with a digital camera attached to the eyepiece.

3.6.4 Instrumental Color

The color of the sample was measured using a colorimeter (CR-10, Konica Minolta Inc., Tokyo, Japan) and expressed in CIE Lab color space, including lightness (L^*), redness (a^*), and yellowness (b^*).

3.6.5 Viscosity

A rotational-type viscometer (Brookfield RVDV-II, AMETEK Brookfield, Massachusetts, USA) was used to measure the viscosity of the sample, and expressed in centipoise (cP), using the method modified from Garrido et al. (2014).

3.6.6 Proximate Composition

The sample was analyzed for its content of water, fat, protein, ash, and carbohydrates using a standard AOAC method (AOAC, 2023).

3.7 Statistical Analysis

Analysis of variance (ANOVA) and Duncan's new multiple range test (DMRT) at a 95% confidence level were employed using SPSS statistical software. All experiments were performed in triplicate. A Completely Randomized Design (CRD) was used for all experiments.

4. Results and Discussion

4.1 Effect of CMC Concentration on Qualities of Plant-based Salad Dressing

Plant-based salad dressings were fabricated using aquafaba as an egg replacer. The AOAC analyzed result showed that the aquafaba used in this study contains approximately 95% water and 5% dry matter. The dry matter of the sample comprised approximately 73% carbohydrate, 13% ash, 7% protein, and 7% fat. These findings are consistent with the report by He et al. (2021a), which found that aquafaba consists of 92–95% water and 5–8% dry matter. Furthermore, previous studies have indicated that the dry matter consists of carbohydrates (sugars and fibers), low molecular weight proteins, saponins, and Maillard reaction products. Similarly, Sahin et al. (2024) reported that the dry matter of aquafaba

contains approximately 76% carbohydrates and 16% protein. Variations in the composition of aquafaba could be influenced by bean cultivar, harvest conditions, climate, and processing parameters, including cooking temperature and the water-to-bean ratio. In addition, CMC was used as a stabilizer in order to improve the stability of plant-based salad dressing. The effects of CMC concentration on ζ -potential and ESI of plant-based salad dressings were investigated in comparison to the control salad dressing (prepared with egg yolk). Figure 1 shows ζ -potential of control salad dressing and plant-based salad dressing with different CMC concentrations. The control salad dressing (prepared with egg yolk) had a positive ζ -potential. This could be explained that the salad dressing particles were coated with surface-active components from egg yolk, including lipoproteins, livetins, and phosvitin (Song & McClements, 2021). In contrast, plant-based salad dressing without CMC had an almost neutral ζ -potential. This could be explained that the main emulsifier in plant-based salad dressing without CMC was chickpea protein from aquafaba, which had ζ -potential in the range of 0.3 to -2.5 mV at pH of 3 to 8.5 (Buhl et al., 2019). However, the plant-based salad dressing containing 0.2 and 0.3 wt% CMC had a negative ζ -potential. This was due to the CMC having a negative ζ -potential, attributed to the carboxyl group (COO^-) in the molecule for a wide range of salt concentrations (0 to 6 %) (Khaled & Abdelbaki, 2012). In addition, ζ -potential indicates the charge of emulsion particles, which affects the interactions between colloidal particles and predicts the stability of emulsion or salad dressing. For example, negatively charged colloidal particles attract positively charged colloidal particles and cations, and vice versa (McClements, 2005; 2015).

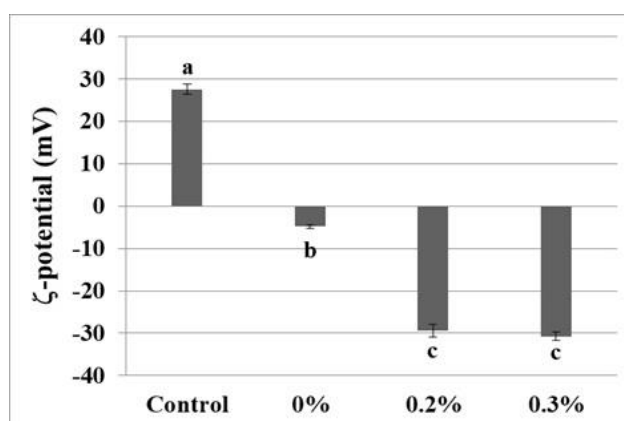


Figure 1 The ζ -potential of control salad dressing and plant-based salad dressing with different CMC concentration

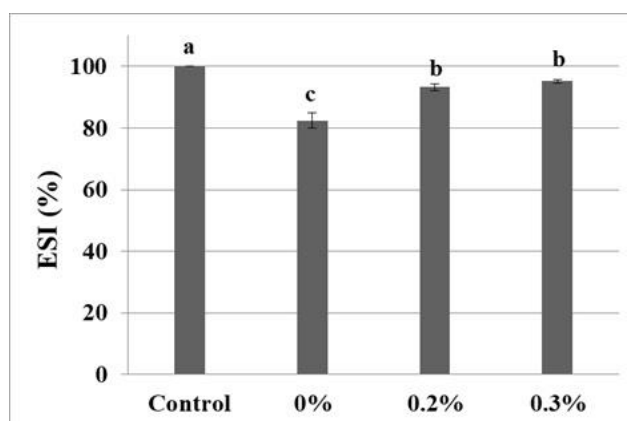


Figure 2 ESI of control salad dressing and plant-based salad dressing with different CMC concentration

ESI of control salad dressing from egg yolk and plant-based salad dressing with different CMC concentrations is shown in Figure 2. The control salad dressing (prepared from egg yolk) and the plant-based salad dressing with 0.2–0.3 wt% CMC were highly stable, with more than 90% ESI. This is consistent with the ζ -potential results (Figure 1), which showed that particles with a ζ -potential magnitude around ± 30 mV or higher are more stable against destabilization mechanisms due to the electrostatic stabilization mechanism (McClements, 2005; Mounsey et al., 2008). Figure 2 also shows that the ESI of plant-based salad dressing with CMC is significantly higher than that of plant-based salad dressing without CMC. This corresponds with Feng et al. (2024), who found that the addition of CMC significantly improved the ESI of the emulsion. The plant-based salad dressing stabilized with 0.2 wt% CMC was selected for the next part of the experiment because it exhibited the highest stability among the plant-based salad dressings with the lowest CMC concentration.

4.2 Effect of Oil: Aquafaba Ratio on Qualities of Plant-based Salad Dressing

The reduced-fat plant-based salad dressings were fabricated in this part of the experiment in order to produce healthier plant-based salad dressings with lower calories. The control sample in this part of the experiment was plant-based salad dressing with an oil: aquafaba ratio of 60:20, stabilized with 0.2 wt% CMC from the previous part of the experiment. The reduced-fat plant-based salad dressings were prepared by reducing the oil ratio while increasing the aquafaba ratio. The effect of the oil: aquafaba ratio on microstructure, droplet diameter, and ESI of plant-based salad dressing was investigated. Figure 3 shows that the microstructure of plant-based salad dressing has small oil droplets dispersed throughout the continuous aqueous phase. The main constituent of the dispersed phase was rice bran oil, whereas the main constituents of the continuous phase included aquafaba, vinegar, and water-soluble substances such as CMC, sugar, and salt, which could not be seen through optical microscopy (Guimarães et al., 2018).

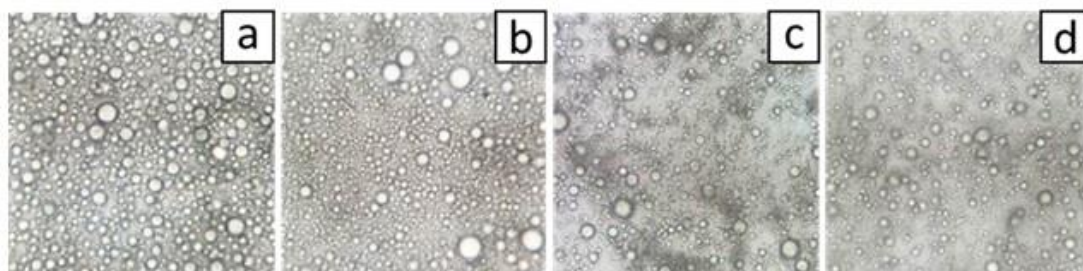


Figure 3 Microstructure of plant-based salad dressing with oil: aquafaba ratio of 60:20 (a), 45:35 (b), 30:50 (c), and 15:65 (d)

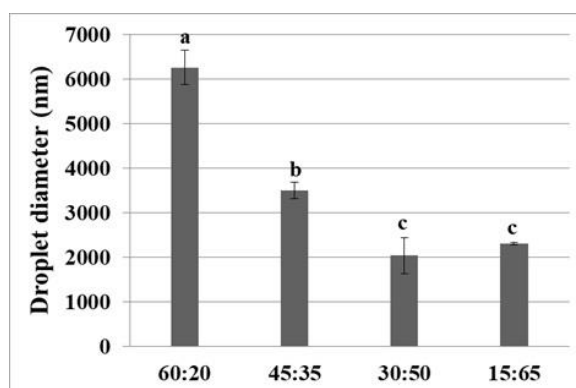


Figure 4 Droplet diameter of plant-based salad dressing with different oil: aquafaba ratios

The mean droplet diameter of plant-based salad dressing was investigated to obtain more details about the droplet size of plant-based salad dressing with different oil: aquafaba ratios. Moreover, droplet size also impacts various characteristics and properties of salad dressing, including viscosity, mouthfeel, turbidity, and stability to gravitational separation (McClements, 2005; 2015). Figure 4 shows the effect of the oil: aquafaba ratio on the mean droplet diameter of plant-based salad dressing. The plant-based salad dressing with the oil: aquafaba ratio of 60:20 had the largest mean droplet diameter, followed by the plant-based salad dressing with the oil: aquafaba ratio of 45:35. However, the plant-based salad dressing with the oil: aquafaba ratio of 30:50 and 15:65 had the smallest mean droplet diameter. The decrease in the mean droplet diameter of plant-based salad dressing as the aquafaba ratio increased could be attributed to the reduction in the oil ratio, which was the main constituent of the disperse phase. The results were similar to Sinsuwan (2024), who found that reduced-fat salad dressing had a mean particle size diameter smaller than full-fat salad dressing. This was supported by the optical micrographs (Figure 3), which showed that the plant-based salad dressing with a lower oil ratio had smaller oil droplets compared to the plant-based salad dressing with a higher oil ratio.

The ESI of plant-based salad dressing with different oil: aquafaba ratios is shown in Figure 5. The

plant-based salad dressings with the oil: aquafaba ratio of 60:20 and 45:35 had the significantly highest ESI, followed by plant-based salad dressing with the oil: aquafaba ratio of 30:50 and 15:65, respectively. This could be explained by the viscosities of plant-based salad dressing with oil: aquafaba ratio of 60:20, 45:35, 30:50, and 15:65, which were $3,760 \pm 226.27$, $1,655 \pm 7.07$, 547.5 ± 21.92 , and 129 ± 1.41 cP, respectively. The salad dressing with a lower oil ratio had lower viscosity due to its lower dispersed phase, resulting in easier phase separation and lower stability than salad dressing with a higher oil ratio (Sinsuwan, 2024). However, the ESI of plant-based salad dressing with oil: aquafaba ratio of 45:35 did not significantly differ from that of plant-based salad dressing with the oil: aquafaba ratio of 60:20, although there were significant differences in droplet diameter and viscosity. This was correspondence with Abdolmaleki et al. (2016), who found that emulsions with significantly different droplet diameters had no significant difference in ESI. This could be explained that emulsion stability is affected by many parameters means, making it difficult to quantify the effect on emulsion properties. The plant-based salad dressing with the oil: aquafaba ratio of 45:35 was selected for the next part of the experiment because it was the most stable plant-based salad dressing with the lowest oil ratio.

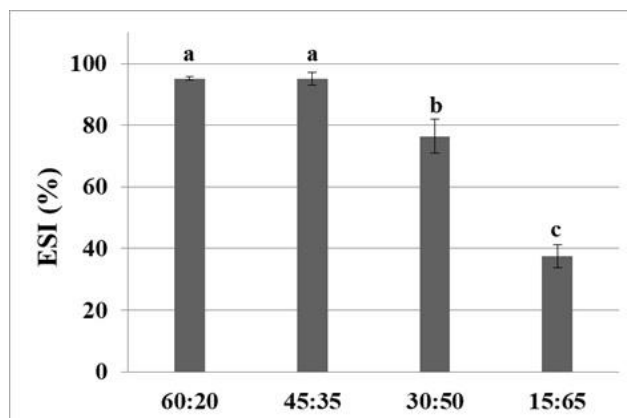


Figure 5 ESI of plant-based salad dressing with different oil: aquafaba ratio

Table 2 Colorimetric parameters of plant-based salad dressing with and without Wolffia

Wolffia concentration (wt%)	L*	a*	b*
0	76.30 ± 0.35 ^a	11.10 ± 0.95 ^a	25.43 ± 3.66
0.15	75.10 ± 1.04 ^{ab}	-11.53 ± 2.55 ^b	23.83 ± 0.59
0.30	73.80 ± 1.01 ^{bc}	-13.37 ± 0.58 ^b	23.63 ± 0.95
0.45	72.43 ± 0.23 ^c	-13.90 ± 0.17 ^b	23.63 ± 0.64

^{a-c} Indicates the significant difference between treatments, $P \leq 0.05$

4.3 Effect of Wolffia Concentration on Qualities of Plant-based Salad Dressing

In this part of the experiment, Wolffia was fortified into plant-based salad dressing to produce a functional plant-based salad dressing with enhanced health benefits. The effect of Wolffia concentration on colorimetric parameters and ESI of plant-based salad dressing was investigated. Table 2 shows the colorimetric parameters (lightness, L^* ; redness, a^* ; and yellowness, b^*) of plant-based salad dressing with and without Wolffia. The L^* values of plant-based salad dressing significantly decreased as the Wolffia concentration increased. This could be due to Wolffia having a dark green color with a low L^* value (Mirón-Mérida et al., 2024); therefore, it decreased the lightness of plant-based salad dressing. The plant-based salad dressing with Wolffia had a positive value of a^* value, while the plant-based salad dressing without Wolffia had a negative value of a^* value. This could be because the green color of Wolffia altered the plant-based salad dressing color from a pale red tone to a slightly green tone. This was correspondence with On- Nom et al. (2023), who found that the addition of Wolffia powder resulted in a green-colored snack with negative a^* values. However, the addition of Wolffia up to 0.45 wt% did not significantly affect the b^* values of plant-based salad dressing.

The ESI of plant-based salad dressing with and without Wolffia is shown in Figure 6. The plant-based salad dressing without Wolffia had the significantly highest ESI, followed by plant-based salad dressings with 0.15 and 0.3 wt% Wolffia, respectively. In addition, the plant-based salad dressing with 0.45 wt% Wolffia had the significantly lowest ESI. This could be explained by the ζ -potential of plant-based salad dressing without Wolffia, and plant-based salad dressing with 0.15, 0.30, and 0.45 wt% Wolffia, which were -37.25 ± 1.60 , -32.81 ± 0.69 , -22.50 ± 0.24 , and -7.64 ± 0.95 mV, respectively. Emulsions with the ζ -potential magnitude less than ± 30 mV are less stable to destabilization mechanisms due to their low electrostatic repulsion between particles (McClements, 2005; Mounsey et al., 2008). Therefore, an increase in Wolffia concentration led to a decrease in the ESI of the plant-based salad dressing. Based on these results, Wolffia should not be added to plant-based salad dressing at levels greater than 0.3 wt% in order to maintain a relatively stable emulsion. In addition, the reduced-fat plant-based salad dressing containing 0.3 wt% Wolffia was analyzed for chemical composition using the AOAC method. The results showed that reduced-fat plant-based salad dressings containing 0.3 wt% Wolffia consisted of approximately 55% fat, 30% water, 13.5% carbohydrate, 1% ash, and 0.5% protein.

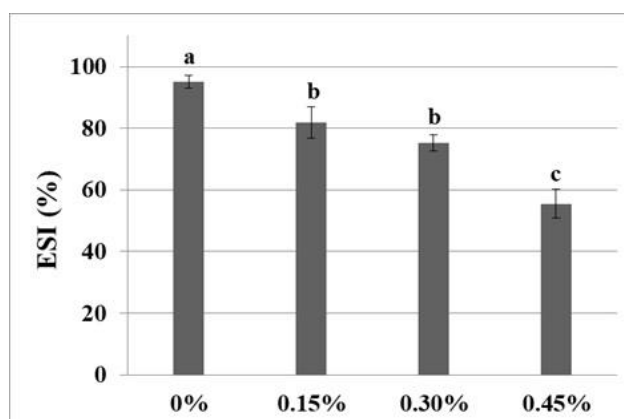


Figure 6 ESI of plant-based salad dressing with and without Wolffia

5. Conclusion

The control salad dressing (prepared from egg yolk) and the plant-based salad dressing with 0.2–0.3 wt% CMC were highly stable with more than 90% ESI owing to their strong electrostatic repulsion and relatively high ζ -potential. The fat content in the plant-based salad dressing could be reduced from 60 to 45 wt% by replacing it with chickpea aquafaba without significantly affecting its ESI. Moreover, reduced-fat plant-based salad dressing with 45 wt% oil had a mean droplet diameter significantly smaller than that of full-fat plant-based salad dressing with 60 wt% oil. In addition, Wolffia fortification significantly reduced the lightness and redness of plant-based salad dressing, but did not significantly affect its yellowness. Unfortunately, Wolffia fortification significantly decreased ζ -potential of plant-based salad dressing, resulting in decreased ESI. The findings from this research could be used for further studies to produce reduced-fat plant-based salad dressing fortified with Wolffia or other nutrients. Furthermore, the effect of Wolffia fortification on the organoleptic quality of reduced-fat plant-based salad dressing should be investigated in future research.

6. Acknowledgements

The authors express their gratitude to the Research Institute of Rangsit University, Rangsit University, Thailand, for financial support. The authors sincerely thank the Food Processing Pilot Plant, and the Faculty of Food Technology, the College of Agricultural Innovation and Food Technology, Rangsit University, for equipment support.

7. CRediT Statement

Panusorn Hunsub: Formal analysis, Data curation, Validation, Visualization, Writing, review & editing.

Pornnapat Khumpagpli: Laboratory Experiments, Investigation, Statistical analysis, Writing, original draft.

Saranporn Srisomsak: Laboratory Experiments, Investigation, Statistical analysis, Writing original draft.

Nut Thephuttee: Methodology, Validation, Writing, review & editing, Supervision.

Tarit Apisittiwong: Methodology, Validation, Resources, Writing, review & editing.

Pitchaya Pothinuch: Investigation, Validation.

Varaporn Laksanalamai: Resources, Funding acquisition.

Nattapong Prichapan: Conceptualization, Validation, data curation, Statistical analysis, Resources, Writing, review & editing, Supervision, Funding acquisition, Project administration.

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