# การประยุกต์ใช้นาโนไฟบริลเซลลูโลสที่สกัดจากเปลือกมังคุดเป็นอิมัลซิไฟเออร์เชิงเดี่ยวในมายองเนส

# APPLICATION OF NANOFIBRILLATED CELLULOSE EXTRACTED FROM MANGOSTEEN RIND AS A SINGLE EMULSIFIER IN MAYONNAISE

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## บทคัดย่อ

วัตถุประสงค์ของการศึกษานี้คือการพัฒนามายองเนสรูปแบบใหม่โดยใช้ NFC เป็นอิมัลซิไฟเออร์เชิงเดี่ยว โดยเตรียมนาโนไฟบริลเซลลูโลส (NFC) จากการสกัดเปลือกมังคุด (*Garcinia mangostana* L.) ด้วยสารละลายโซเดียมไฮดรอกไซด์ที่ร้อนจากนั้นเฉือนด้วยโฮโมจีไนเซอร์ความดันสูงในการผลิตมายองเนส ไข่แดงถูกแทนที่ด้วย NFC ความเข้มข้นต่างๆ (5-10%) และมีการวัดเนื้อสัมผัส สี pH ขนาดของอนุภาค สมบัติเชิงรีโอโลยี และการทดสอบด้านประสาทสัมผัส ผลการทดลองปรากฏว่าหยดน้ำมันของมายองเนสสูตร NFC มีเส้นผ่าศูนย์กลางใหญ่กว่าสูตรไข่แดง เมื่อความเข้มข้นของ NFC เพิ่มขึ้นส่งผลให้เส้นผ่าศูนย์กลางของหยดน้ำมันและค่าความสว่างของมายองเนสลดลงในขณะที่ความหนืดเพิ่มขึ้น มายองเนสทุกสูตรแสดงลักษณะโครงสร้างคล้ายเจลและมีพฤติกรรมแบบ Thixotropic Shear Thinning เมื่อเก็บไว้ที่อุณหภูมิ 25 องศาเซลเซียส เป็นเวลา 90 วัน พบว่าไม่มีการแยกชั้น นอกจากนั้นมายองเนสสูตร NFC ยังได้รับการยอมรับจากผู้ร่วมทดสอบทางประสาทสัมผัสที่ชอบเล็กน้อยถึงปานกลาง

**คำสำคัญ:** นาโนไฟบริลเซลลูโลส เปลือกมังคุด มายองเนส อิมัลซิไฟเออร์

## Abstract

The aim of the study was to develop the new formulation of mayonnaise using this NFC as a single emulsifier. Nanofibrillated cellulose (NFC) was prepared by extraction of ground mangosteen (*Garcinia mangostana* L.) rind using hot sodium hydroxide solution and subsequent shearing using a high-pressure homogenizer. Egg yolk was replaced with NFC at different levels (5-10%) for the formulated mayonnaises. The texture, color, pH, particle size, rheology, and sensory liking scores were determined. The result found that the NFC concentration affected the physicochemical properties of the mayonnaise. The oil droplet diameter of the NFC mayonnaises was larger than those of the egg yolk mayonnaise. The oil droplet diameter and lightness values decreased with increasing NFC concentration ,but on the contrary, the apparent viscosity increased. All mayonnaises exhibited gel-like structure and thixotropic shear thinning behavior. Additionally, no cream and serum layer separation were observed in all formulas after storage at 25°C for 90 days. Moreover, all NFC mayonnaises were accepted by panelists with the sensory liking scores between like slightly and like moderately.

**Keywords**: Nanofibrillated Cellulose, Mangosteen Rind, Mayonnaise, Emulsifier

## Introduction

Mayonnaise is one of the most common foods used all around the world. It is a stable oil-in-water emulsion of oil, egg yolk, and acid, either vinegar or lemon juice, with some herb, spice or salt. Among mayonnaise ingredients, egg yolk is normally used as an emulsifier and also the most critical for the stability of the product [1]. The main problem of using egg yolk is its high cholesterol content. Moreover, egg yolk does not suitable for vegetarian consumer and it can be considered as an allergen.

The common emulsifiers used in food-based emulsion manufacturing, such as monoglycerides, Spans, Tweens, are considered as a synthetic emulsifier [2-3]. In many countries, lecithin (vegetable lecithin or animal lecithin) is an emulsifier that probably the only commercially available food-grade from natural, although great efforts have been spent to seeking for a new food-grade emulsifier [4-6].

The isolation of cellulose fibril aggregates using a homogenization process has first been described [7]. The resulting cellulose fibers are moderately degraded and opened into their substructural fibrils and microfibrils, which is called “nanofibrillated cellulose (NFC) or microfibrillated cellulose (NFC)”. Nowadays, the nanocelluloses were allowed to use in commercial as a stabilizer, rheology modifier and packaging [8]. The NFC is generally produced from many sources such as the cellulosic residues and wastes produced from agricultural and industrial activities. Many study was obeserved new sauces of nanocellulose, such as banana peel [9], banana rachis, sisal, kapok, pineapple leaf, coir [10], rice straw [11-12], miez straw [13], and lotus leaf stalk [14]. These materials provided the nanocellulose with diameter between 2-150 nm [9-14] depending on material and preparation method used [15].

In this study, NFC was prepared by extraction of ground mangosteen (*Garcinia mangostana* L.) rind with hot alkali pre-treatment and mechanical force using a high-pressure homogenizer. Mangosteen rind was chosen to be used as a raw material because it is about two-thirds of the whole fruit weight and also usually disposed of agricultural waste [16]. These wastes are rich in cellulose about 60-70% and have been used for the production of NFC [17]. Previous studies have shown the emulsifying properties of NFC in an oil-in-water emulsions by absorbing at oil-water interface of emulsion. This stabilizing mechanism is called “Pickering mechanism” [17-18]. For this reason, we expected that this NFC could be used as a single emulsifier to replace egg yolk in emulsion-based food product such as mayonnaise. The use of this NFC to completely replace egg yolk in the formulation has the four-fold advantages of (1) reducing the cholesterol content in the product, because of replacing egg yolk in the formulation, (2) increasing dietary fiber content in the product, (3) utilizing products derived from industrial by-product, and (4) obtaining the new type of mayonnaise which is suitable for health conscious people who are concerned about their cholesterol intake and/or who are vegetarian. In fact, there is no published data in the literature on functions of NFC as an emulsifier in mayonnaise.

## Objectives

The aim of the study was to develop new formulations of mayonnaise using NFC as a single emulsifier and then the physicochemical properties, rheology, and sensory liking scores of the formulated mayonnaise were determined.

## Methods

### Materials

Dried mangosteen (*Garcinia mangostana* L.) rind, a by-product of the mangosteen-canning process, was supplied by a local manufacturer (Chanthaburi, Thailand). Sodium hydroxide (NaOH) and phosphoric acid ($H_3PO_4$) were purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). Hydrogen peroxide ($H_2O_2$) was purchased from Merck KGaA (Darmstadt, Germany). Tumeric extracted powder, vinegar, lime juice, egg yolk, mustard, sugar, salt, and oil were purchased from a local supermarket.

### Preparation of NFC

Nanofibrillated cellulose (NFC) from mangosteen rind was prepared according to the method was described [16]. Briefly, the dried mangosteen rind was grounded through a 100 mesh sieve. The cellulose was extracted using hot NaOH solution (90°C) at pH 12, washed, neutralized, and then bleached using hot $H_2O_2$ solution (90°C). The yellow-brown, water-swollen purified cellulose was collected by filtration. The purified cellulose contained protein and fat less than 1% (w/w), while carbohy drate, total dietary fiber, insoluble dietary fiber and cellulose more than 90% (w/w) [17]. The purified cellulose was used for the preparation of NFC by re-dispersing in distilled water at a concentration of 1% w/w and then passing through a high pressure homogenizer (APV® 1000, SPX Flow Technology Crawley Ltd., West Sussex, UK) at a pressure of 500 bar for 20 passes at room temperature (25°C).

The appearance of final material was yellow-brown gel-like aqueous matter (pH 7) having the NFC concentration of 1% w/w.

**Preparation of Mayonnaise**

Mayonnaise formula was slightly modified from mayonnaise which was used β-glucan as a fat replacer [19]. The mayonnaise ingredients were showed in Table 1. In this experiment, for the NFC mayonnaises, egg yolk was completely replaced by NFC at levels of 5%, 7.5%, and 10%. All ingredients, except oil, were mixed gently by a hand blender (BOSH® MSM67160, Robert Bosch GmbH, Gerlingen, Germany) at 3,200 rpm for 5 minutes. Then, the oil was gradually added and mixed continuously at 3,200 rpm for 2 minutes. The prepared mayonnaises were packed in a polypropylene cup and covered with a lid. The samples were stored at 4°C for 1 day before testing.

**Table 1** Mayonnaise recipes (g/100g)

| Formula / Ingredient | Control | NFC Concentration (%) | | |
|---|---|---|---|---|
| | | 5.0 | 7.5 | 10.0 |
| Vinegar | 4.00 | 4.00 | 4.00 | 4.00 |
| Lime Juice | 4.00 | 4.00 | 4.00 | 4.00 |
| NFC | - | 5.00 | 7.50 | 10.00 |
| Egg Yolk | 10.00 | - | - | - |
| Mustard | 0.36 | 0.36 | 0.36 | 0.36 |
| Sugar | 3.00 | 3.00 | 3.00 | 3.00 |
| Salt | 0.71 | 0.71 | 0.71 | 0.71 |
| Tumeric Powder | 0.05 | 0.05 | 0.05 | 0.05 |
| Oil | 77.88 | 82.88 | 80.38 | 77.88 |

**pH Measurement**

The pH of mayonnaise was measured using a pH meter (Starter 2100, Ohaus Corp., New Jersey, USA) at a room temperature (25°C).

**Color Measurement**

The color of the mayonnaise was measured in the *L**, *a**, *b** system using Hunter lab color Flex® (EZ, Hunter Associates Laboratory Inc., Virginia, USA). *L** represents the lightness, and *a** and *b** are color coordinates: where +*a* is red, -*a* is the green, +*b* is yellow, and -*b* is the blue directions.

**Creaming Stability Measurement**

Creaming stability was measured using a protocol of Guerra-Rosas with minor modification [20]. Ten grams of mayonnaise were transferred to a glass tube and sealed with plastic cap. Then, the tube was stored at room temperature (25°C) for 90 days. After storage, the emulsion was normally separated into two layers, including an optically opaque "cream layer" at the top and a transparent (or turbid) "serum layer" at the bottom. In everyweek, the total height of the emulsion ($H_E$) and the height of the serum layer ($H_S$) were measured. The extent of creaming was calculated by the following equation.

Creaming index (%) = 100 X ($H_S$/$H_E$).

The creaming index provided indirect information about the extent of droplet aggregation in an emulsion, such as the faster creaming and the larger particle size of oil droplets, the higher creaming index.

**Particle Ssize Measurement**

The particle size of the sample was analyzed by a particle size analyzer (Mastersizer 3000, Malvem Panalytical Ltd, Malvern, UK). Droplet size measurements were reported as the volume-weighted mean diameter ($d_{4,3}$) and surface-weighted ($d_{3,2}$) mean particle diameter.

**Texture Measurement**

Texture measurement was carried out using a Texture Analyzer (TA.XT plus, Stable Micro Systems Ltd, Surrey, UK). The aluminum cylinder probe (p/25) was used. The probe height calibration was set to return distance at 50 mm, return speed at 10 mm/sec, and contact force of 5 g. The sequence menu was set pre-test speed at 1 mm/sec, test speed at 2 mm/sec, and post-test speed at 5 mm/sec with a distance and time of 20 mm and 5 sec, respectively. A plastic cylinder cup (diameters 55 mm, high 60 mm) was used as a container and each cup contained 70 g of the mayonnaise. From the resulting force-time curve, the values for texture attributes, such as hardness and adhesiveness, were obtained using the Texture Expert for window equipment software.

**Rheological Measurement**

The rheology measurement was performed using a rheometer (HAAKE™ MARS 400, Thermo Fisher Scientific Inc., Massachusetts, USA) with a cone and plate (35 mm diameter, 2° cone angle) at 25°C. The linear viscoelastic range was determined with strain sweep (0.01-100%) at a fixed frequency of 10 rad/s. Then, the dynamic frequency sweep was conducted by applying a constant strain of 0.1% of which was within the linear region, over a frequency range between 0.1 and 100 rad/s. The mechanical spectra were obtained recording as dynamic moduli $G'$ (storage modulus), $G''$ (loss modulus).

The steady flow test was also performed. The shear rate increased continuously up to 300 $s^{-1}$ followed by a shear rate in a decreasing mode from 300 $s^{-1}$ to 0.1 $s^{-1}$ in 6 minutes, the former is referred to as 'up' curves and the latter as 'down' curves.

**Sensory Evaluation**

Sensory evaluation of freshly prepared mayonnaise was conducted. Sensory characteristics, such as appearance, texture, flavor, and overall liking scores were evaluated by 40 untrained panelists, who aged between 18-60 years old and normally consume mayonnaise product, using 9-point hedonic scale (1 = dislike extremely, and 9 = like extremely). Panelist evaluated all four samples four times each, once in each session. Presentation order was randomized for each session and between panelists. The sample was served with boiled chicken breast and carrot. Drinking water was provided to panelists between the samples to cleanse the palate.

**Statistical Analysis**

A one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test ($p \leq 0.05$) were used to establish the significant difference of the physicochemical properties and sensory characteristic of all samples. The analyses were performed using the SPSS version 19.0 for window program (SPSS Inc., Chicago, IL, USA).

## Results

**pH and Color Measurement**

The pH values of the mayonnaise samples were measured. The results (Table 2) show that the pH value of all NFC formulas were significantly ($p \leq 0.05$) lower than that of the control. For color (Table 2), the $L^*$, $a^*$ and $b^*$ values of NFC formulas were significantly ($p \leq 0.05$) lower than those of the control. The $L^*$ and $b^*$ values decreased with increasing level of NFC, while the $a^*$ values were increased.

**Physical Properties**

The creaming index was not shown because there is no separation occurred throughout the experiment (Fig 1). Table 3 shows the oil droplet diameter of the mayonnaise. There was a decrease in both $d_{3,2}$ and $d_{4,3}$ when the NFC concentration was increased from 5 to 10%. For each formula, the $d_{4,3}$ value was much higher than the $d_{3,2}$ value. The hardness showed 5% of NFC formula were significantly softer than 10%, 7.5%, and control respectively. For an adhesiveness, all of the formulas were not significantly different.

**Rheology Properties**

Small amplitude oscillatory shear measurement was performed to determine viscoelastic properties of the formulated mayonnaises. The mechanical spectra of all mayonnaise formulas show that $G'$ was higher than $G''$ with no crossover throughout the tested frequency range (Fig 2A). This phenomenon is typically known as gel-like behavior [20]. The magnitude of $G'$ and $G''$ were not depended on NFC concentration. The results show that the 7.5% NFC mayonnaise exhibited the highest $G'$ among all tested NFC mayonnaises, indicating the highest in the strength of emulsion microstructure. The NFC at 7.5% may be the suitable amount of emulsifier used for stabilizing the amount of oil used in this mayonnaise formula. For the steady shear measurement, flow curves of all tested mayonnaises exhibited mainly time-dependent shear-thinning (thixotropic) behavior in the shear rate range tested (Fig 2B).

The flow behavior of the NFC mayonnaises showed yield stress behavior, which the presence of spurs at low shear rates on the upward flow curves, whereas the control did not. The apparent viscosity of all formulas decreased with increasing the shear rate and increased with NFC concentration (Fig 2C), also indicating shear-thinning behavior.

**Sensory**

The results of sensory evaluation of the mayonnaise using NFC as an emulsifier at different levels compared with the egg yolk mayonnaise are shown in Table 4. The appearance, texture and overall acceptability scores of the NFC formulas were significantly lower than those of the control.

**Table 2** pH and color of mayonnaise using egg yolk (control) and NFC as a single emulsifier.

| | | Color | | |
|---|---|---|---|---|
| **Sample** | **pH** | ***L**** | ***a**** | ***b**** |
| Control | 3.08±0.05[a] | 84.62±0.82[a] | 6.54±0.02[a] | 33.25±0.17[a] |
| 5% NFC | 1.90±0.04[c] | 69.37±0.13[b] | 2.24±0.05[d] | 27.14±0.20[b] |
| 7.5% NFC | 1.93±0.02[bc] | 69.43±0.12[b] | 3.07±0.10[c] | 25.05±0.12[d] |
| 10% NFC | 1.97±0.02[b] | 68.26±0.31[c] | 4.02±0.11[b] | 25.56±0.12[c] |

Mean ± S.D. value followed by letter each column are significantly different at $p \leq 0.05$ by ANOVA and Duncan's Multiple Range Test.

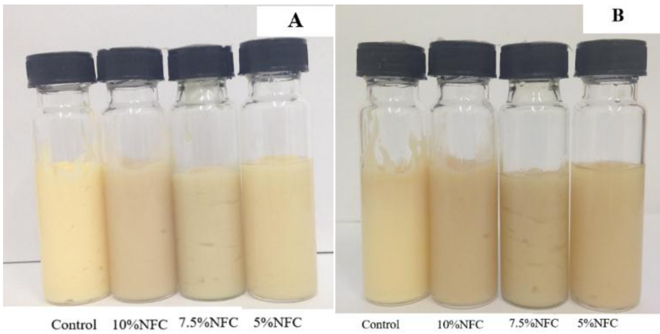


**Figure 1** Optical images of mayonnaise using egg yolk (control) and NFC as a single emulsifier storage at 25°C for 1 day (A) and 90 days (B).

**Table 3** Surface-weighted ($d_{3,2}$) and volume-weighted ($d_{4,3}$) mean diameter, hardness, and adhesiveness of mayonnaise samples

| | Particle size (μm) | | Texture | |
|---|---|---|---|---|
| **Sample** | $d_{4,3}$ | $d_{3,2}$ | **Hardness (g)** | **Adhesiveness (g.sec)** |
| Control | 3.53±0.29[C] | 2.04±0.18[b] | 243.08±19.43[a] | -673.51±468.26[a] |
| 5% NFC | 75.31±3.06[a] | 28.31±2.60[a] | 100.51±11.82[c] | -307.21±121.12[a] |
| 7.5% NFC | 74.72±4.21[b] | 28.27±4.18[a] | 129.22±0.58[b] | -471.26±49.28[a] |
| 10% NFC | 65.57±4.33[b] | 25.93±2.84[a] | 121.87±4.03[bc] | -427.72±76.22[a] |

Mean ± S.D. value followed by letter each column are significantly different at $p \leq 0.05$ by ANOVA and Duncan's Multiple Range Test.

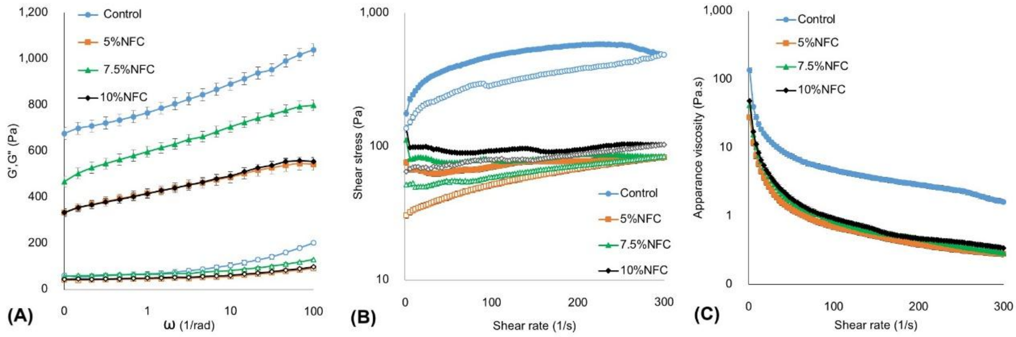


**Figure 2** Influence of NFC concentration on (A) mechanical spectra, i.e. storage modulus, $G'$, (closed symbols) and loss modulus, $G''$, (open symbols) as a function of angular frequency, (B) flow curves, *i.e.* shear stress as a function of shear rate, in which closed symbols represent the upward curves and open symbols represent the downward curves, and (C) apparent viscosity as a function of shear rate of fresh control (egg yolk) and NFC mayonnaises. All measurements were performed at 25°C.

**Table 4** Sensory scores of egg yolk (control) and NFC mayonnaise using the 9-point hedonic scale.

| Sample | Appearance | Flavor | Texture | Overall Acceptability |
|---|---|---|---|---|
| Control | $7.37 \pm 1.26^{a}$ | $7.08 \pm 1.53^{a}$ | $7.32 \pm 1.36^{a}$ | $7.21 \pm 1.4^{a}$ |
| 5% of NFC | $6.76 \pm 1.22^{b}$ | $6.08 \pm 1.40^{b}$ | $6.46 \pm 1.23^{b}$ | $6.54 \pm 1.22^{b}$ |
| 7.5% of NFC | $6.26 \pm 1.41^{b}$ | $6.37 \pm 1.17^{b}$ | $6.61 \pm 1.15^{b}$ | $6.45 \pm 1.16^{b}$ |
| 10% of NFC | $6.47 \pm 1.33^{b}$ | $6.47 \pm 1.45^{ab}$ | $6.50 \pm 1.31^{b}$ | $6.55 \pm 1.31^{b}$ |

Mean ± S.D. value followed by letter each column are significantly different at $p \leq 0.05$ by ANOVA and Duncan's Multiple Range Test.

## Conclusions and Discussion

This study focused on developing non-egg mayonnaise by using NFC as a single Pickering emulsifier. The concentration of NFC exhibited a huge influence on properties of the mayonnaise, including pH, color, hardness, and rheological propertied. These properties and creaming index effected to stability of the formulated mayonnaise products. The NFC used in this study was an isolated cellulose fiber extracted from original cellulose extracted from mangosteen rind by using hot alkaline extraction in combination with homogenization process. The pH and hardness increased when the concentration of NFC used was increased from 5 to 10%, whereas the lightness and particle size was decreased. The concentration of NFC also affected the gel strength, shear stress, and apparent viscosity. At least 7.5% NFC was needed to form and stabilize the mayonnaise with concerning the texture and rheological properties together. However, no phase separation was observed for all tested mayonnaises after storage at 25°C for 90 days.

The concentration of NFC affected pH because of the dilution effect due to NFC contained 99% of water. In addition, NFC exhibited pH7 because the pH of NFC was adjusted during preparation step. Hence, the pH of mayonnaise increased with increasing NFC concentration in the formulas. Similarly, the concentration of NFC also affected color. The mayonnaise containing high concentration of NFC was darker than the one with lower NFC concentration. The color of the mayonnaise was changed with increasing NFC concentration because NFC itself had a yellow-brown color ($L^*$ = 31.73, $a^*$ = 0.74, $b^*$ = 8.37).

For droplets size, the difference of $d_{3,2}$ and $d_{4,3}$ that they are sensitive to different aspects of the particle size distribution, i.e. $d_{3,2}$ is more sensitive to the presence of small particles, whereas $d_{4,3}$ is more sensitive to the presence of large particles in the size distribution [2]. Our results were not in the same agreement [18], they found that the oil droplet diameter increased with increasing the concentration of microfibrillated cellulose because the higher viscosity of the continuous phase may hinder the emulsifier movement during emulsification and their final deposition at the oil-water interface affected to viscosity was increased that interfered to homogenizer ability. However, the other studies reported that when the concentration of emulsifier was increased, the droplet size decreased. In addition, at high NFC concentration, the NFC mostly formed three-dimension network which helped to stabilize and prevent the oil droplets from coalescence [22-26].

The concentration of NFC influenced the structure of the mayonnaise because NFC itself could form the three-dimensional network, thereby affecting the strength and hardness of samples [26-28]. From the results of flow behavior, the yield stress related to the strength of the three-dimensional network structure that must be broken down at higher shear rates to cause flow [29]. The result indicating shear-thinning behavior. The shear-thinning behavior is a typical characteristic of an emulsion containing flocculated droplets, in which the changes in floc size and shape are induced by shearing action [2, 18, 30]. Moreover, in the previous study, we have demonstrated that the microfibrillated cellulose-stabilized emulsions were not susceptible to shear-induced coalescence under the shear conditions applied during the steady shear measurement [17]. Overall, there was a good relationship between the rheology of the mayonnaises determined by both dynamic and steady shear measurements and their texture (hardness) observed using a Texture Profile Analyzer

This sensory evaluation related to the color and texture results. For example, the panelists could detect the color and texture changes in mayonnaise when $L^*$, $a^*$

and *b** value and hardness of NFC formulas were different with control. For overall acceptability, all NFC mayonnaise formulas were accepted by 40 untrained panelists with the score between like slightly to like moderately. The acceptability score of the NFC mayonnaise was lower than that of the control because the NFC formulas were darker, sourer and lower viscous. Although all formulations of NFC mayonnaise were accepted by the panelists at the same level, the physicochemical and rheological properties were different. At least 7.5% NFC was needed to form and stabilize the mayonnaise with concerning both sensory acceptability and rheological properties. In conclusion, this study demonstrates that NFC may have the potential for some applications as an emulsifier in mayonnaise which is suitable for health-conscious people who are concerned about their cholesterol intake and/or who are vegetarian and/or who has an egg allergy. The information obtained from this study can be used as potential applications for further research in other emulsion-based food products.

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