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# The Effects of Heat Treatment on the Stability of Oil-in-Water Nanoemulsions Stabilized by Nanocellulose

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**Abstract.** Oil-in-water nanoemulsions are created by combining oil and water into nanosized mixtures. The stability of these emulsion systems can be significantly affected by processing and storage under uncontrolled temperatures. Investigating the effects of heat treatment and stabilizers on nanocellulose-stabilized oil-in-water nanoemulsions is essential. This study involves three different heating temperatures: 60°C, 70°C, and 80°C. Furthermore, the nanocellulose concentration was altered to 0%, 0.10%, and 0.20%. The findings indicated that elevated temperatures combined with reduced concentrations of nanocellulose diminish the lightness of emulsions. Nevertheless, the distinctions are invisible to human sight. At concentrations 0.20% and 70°C, particle size, and enhanced particle charge were reduced in oil-in-water nanoemulsions, indicating greater electrostatic repulsions. Viscosity escalates with elevated concentrations of the nanocellulose mixture and temperature. Consequently, the creaming index diminished, and the emulsion attained more stability. The amalgamation of NCC and NFC, particularly at 0.20%, serves as a natural stabilizer, enhancing the strength of o/w nanoemulsion to a temperature of 70°C.

**Keywords:** nanoemulsions, nanocrystalline cellulose, nano fibrillated cellulose, heat treatment

## 1. Introduction

An emulsion is a formulation of polar and non-polar solution, one of the phases is distributed finely and uniformly disseminated within the other, typically stabilized by an emulsifying agent. The oil-in-water phase is extensively utilized in the food sector. Oil-in-water emulsions include margarine, mayonnaise, milk, cream, and coconut milk [1]. Nonetheless, emulsion systems are typically unstable. Consequently, emulsifiers and stabilizers are required [2].

Appropriate and adequate emulsifiers and stabilizers are essential for stabilizing the emulsion. Mollet & Grubenmann [3] assert that emulsification requires chemical and physical energy. The emulsification process employs chemical energy by adding an emulsifier, whereas the physical process may utilize temperature or the pace of emulsification. Various emulsifiers exist, including CMC, Tween 20, and Tween 80. The purpose of using an emulsifier is to lower the



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surface tension from the dispersed and dispersing phases [4]. Numerous emulsifiers and stabilizers exist; nevertheless, utilizing natural components, such as nanocellulose, is advisable.

Nanocellulose is categorized as a natural fiber derived from cellulose. Nanocellulose serves as an emulsifier or stabilizer due to its lightweight, biodegradable nanofiber characteristics, including a low density of around 1.6 g/cm<sup>3</sup> and exceptional tensile properties. Nanocellulose is categorized into three distinct types according to its structure. Initially, acid hydrolysis often isolates Nanocrystalline Cellulose (NCC) from cellulose fibrils. The amorphous component is hydrolyzed and eliminated by acid, whereas the crystalline component persists. NCC possesses a width from 3 to 20 nm [5]. Secondly, Nanofibrillated Cellulose (NFC) consists of elongated, flexible, and intertwined nanocellulose that can be generated from cellulose fibrils through mechanical processes. Typically, the diameter is under 100 nm [5]. Third, bacterial cellulose is derived from bacteria, exhibiting a width of around 2-20 nm[6].

Prior research utilized nanocellulose as a stabilizer in an o/w emulsion system, employing only a singular variety of nanocellulose. Research conducted by [7] indicates that nanocrystalline cellulose enhances the stability of the emulsion system. Similarly, using NFC nanocellulose, as shown in [8], stabilized emulsions can diminish the o/w interfacial tension, reducing emulsion droplet diameter and inhibiting coalescence. Limited research has been undertaken on emulsions that utilize heating temperature as a facilitator for the emulsification process.

Emulsions stabilized by nanocellulose exhibit structural stability, resulting in prolonged stability during storage. The emulsion exhibited commendable stability when nanocellulose without surface charge was employed as a stabilizer. Hopefully, it can be used to observe the optimal nanocellulose combination for enhancing the stability of o/w emulsions, given that most food items are subjected to heat treatment during processing.

## 2. Materials and methods

### 2.1 Materials

The equipment included Particle Size Analyzer (PSA) Model Microtac Nanotrac Wave II, a Colorimeter NH310 series, a Magnetic Stirrer, a Homogenizer IKA Model T25 Digital Ultra Turrax, a Viscometer DVE Brookfield series EB21BETA, a Glass Beaker, and a 20 ml Glass Bottle. This research utilized nanocrystalline cellulose (NCC) and nanofibrillated cellulose (NFC), both bought from Cellulose Lab Company, Canada. Tween 20 and sodium azide were acquired from the CV chemical store. Pratama Chem-Mix. Aquades. Palm oil was acquired at Indogrosir in Jogja.

### 2.2 Methods

The oil phase was effectively formulated by measuring 1 gram of soybean oil. Moreover, aqueous phase form from 1% (w/w) solution of Tween 20 and 0.01% (w/w) sodium azide to enhance its antimicrobial properties, and a ten mM sodium phosphate buffer solution was adjusted to a pH of 7. Then, it was efficiently carried out using a high-shear mixer for 2 minutes at ambient temperature (25 °C), ensuring a well-integrated mixture. To refine the emulsion, the coarse mixture underwent further processing with a shear mixer for 5 minutes at a power setting of 50%, employing a pulsed on/off cycle of 5 seconds throughout this time. Following this, the emulsion was allowed to equilibrate at room temperature (25 °C) for one hour.

#### 2.2.1. Visual Creaming Stability

Fresh samples were promptly placed into transparent glass test tubes measuring 15 mm in diameter and 80 mm in height and sealed with plastic caps to ensure their integrity. To minimize external influences, the sample tubes were then maintained at a consistent 25 °C in a dark environment. Then, it systematically monitored the separation of the creaming layer at intervals of 0, 1, 3, and 7 days. This observation allowed us to calculate the creaming index, an important metric for assessing emulsion quality, using the following equation:

$$CI (\%) = \frac{HS}{HT} \times 100 \quad (1)$$

Where HT is the total height of the emulsion in the tube and HS is the height of the creaming layer to be measured.

### 2.2.2. Color

The color measurement of the emulsion was carried out on day 0 using the L\*, a\*, and b\* parameters with a 3NH NH310 colorimeter (Shenzhen Threenh Technology Co., Ltd, China). A 15 ml sample was transferred into an analysis vial, sealed, and then assessed using the colorimeter. In this context, L\* represents brightness, while a\* and b\* denote color coordinates, with a\* corresponding to red and b\* to yellow. The following calculation quantifies the overall color variation:

$$\Delta E = \sqrt{(L^*_{ii} - L^*_i)^2 + (a^*_{ii} - a^*_i)^2 + (b^*_{ii} - b^*_i)^2} \quad (2)$$

Where i is an emulsion sample without the addition of nanocellulose with varying heating temperatures, and ii is an emulsion sample with the addition of nanocellulose and varying heating temperatures at 60 °C, 70 °C, and 80 °C.

### 2.2.3. Particle Size

The particle size and distribution were analyzed by using the Mastersizer 2000 laser diffraction particle size analyzer (Malvern Instruments Ltd., Worcestershire, United Kingdom). Samples were thoughtfully diluted in a 10 mM phosphate buffer solution at pH 7.

### 2.2.4. ζ-Potential

The particle charges were analyzed by using a particle electrophoresis device (Zetasizer Nano ZS, Malvern Instruments Ltd., Worcestershire, United Kingdom). Furthermore, the sample dilution was conducted using 10 mM phosphate buffer at pH 7.

### 2.2.5. Apparent Viscosity Measurement

All samples' viscosity was analyzed using a Brookfield viscometer (PT Alfa Omega Indolab). First, a 50ml sample was added into a beaker glass, then analyzed using torch number 6.

### 2.2.6. Statistical Analysis

All findings from this study were presented as means and standard deviations. However, the significance differences continued by a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test was employed, with significance set at p<0.05. The statistical analysis was performed using SPSS version 25 software.

## 3. Results and Discussions

### 3.1. Color

The emulsion sample () has a milky white hue and maintains a rather thin consistency due to the amalgamation of oil, water, Tween 20, sodium azide, and other nanocellulose components. Table 1 illustrates the impact of heating temperature on the concentration of nanocellulose with respect to color. The results indicated a substantial difference (p<0.05) in the L\* values of samples at identical temperatures but varying concentrations, which diminishes as the concentration of additional nanocellulose increases. In addition to color features, the dimensions of the sample's oil particles might influence the emulsion's hue. L\* is correlated with the particle size of the oil droplet. Emulsions comprise several oil droplet size ranges, resulting in differential scattering of light waves by each droplet size class, with larger droplets capable of absorbing lighter, thereby

diminishing the  $L^*$  value [9].  $L^*$  will diminish when NCC and NFC concentrations rise due to the increased viscosity of the emulsion at elevated NCC and NFC levels.

An increase in concentration is derived in a drop in the  $a^*$  value. At a temperature of 80° C, an increase in nanocellulose concentration correlates with a rise  $a^*$  value. For this  $a^*$  value, the optimal concentration is 0.20%, principally to ensure protection at a temperature of 70° C. As the concentration of nanocellulose increases, the  $b^*$  value decreases. An increased concentration of nanocellulose in the sample resulted in a decrease in the  $b^*$  color value, signifying less yellowness. The total color value  $\Delta E$  exhibits an increase; when the concentration of nanocellulose rises, the total  $\Delta E$  value also escalates. The nanocellulose concentration at 0.70° C has the highest value among all tested temperatures. An emulsion is considered stable if it has uniformly shaped droplets that retain their original color and resist degradation. Upon examining the values of the color parameters for concentration and temperature, a concentration of 0.20% at a temperature of 70° C is determined to be the most stable and effective for heating the emulsion.

Table 1. The effects of temperature and nanocellulose concentration on color ( $L^*$ ,  $a^*$ ,  $b^*$ ) of oil-in-water emulsions.

Concentration	Temp (°C)	$L^*$	$a^*$	$b^*$	$\Delta E$
0%	60	56.83 ± 0.59 <sup>ef</sup>	-0.85 ± 0.02 <sup>a</sup>	1.62 ± 0.16 <sup>a</sup>	-
	70	60.28 ± 0.41 <sup>g</sup>	-0.74 ± 0.01 <sup>b</sup>	1.92 ± 0.09 <sup>b</sup>	-
	80	57.11 ± 0.40 <sup>f</sup>	-0.51 ± 0.01 <sup>fg</sup>	1.74 ± 0.10 <sup>ab</sup>	-
0.10%	60	55.08 ± 0.34 <sup>b</sup>	-0.70 ± 0.09 <sup>bc</sup>	1.83 ± 0.10 <sup>b</sup>	1.82 ± 0.68 <sup>a</sup>
	70	56.01 ± 0.24 <sup>cde</sup>	-0.64 ± 0.02 <sup>cd</sup>	1.87 ± 0.03 <sup>b</sup>	4.27 ± 0.31 <sup>c</sup>
	80	56.15 ± 0.10 <sup>de</sup>	-0.47 ± 0.01 <sup>g</sup>	2.22 ± 0.05 <sup>c</sup>	1.38 ± 0.13 <sup>a</sup>
0.20%	60	54.10 ± 0.24 <sup>a</sup>	-0.56 ± 0.04 <sup>ef</sup>	1.86 ± 0.14 <sup>b</sup>	2.76 ± 0.37 <sup>b</sup>
	70	55.13 ± 0.21 <sup>bcd</sup>	-0.60 ± 0.05 <sup>de</sup>	1.74 ± 0.03 <sup>ab</sup>	5.16 ± 0.56 <sup>d</sup>
	80	55.37 ± 0.56 <sup>bc</sup>	-0.72 ± 0.07 <sup>bc</sup>	1.78 ± 0.08 <sup>ab</sup>	1.20 ± 0.42 <sup>ab</sup>

Note: The test was carried out in three repetitions. The same column followed by different lowercase letters (a-g) indicates significant differences ( $p < 0.05$ ).

This is due to the structural characteristics of nanocellulose, which exhibits remarkable solubility in water or hydrophilic qualities, attributable to the abundance of hydroxyl groups capable of forming hydrogen bonds with water. However, this is inaccurate as nanocellulose is insoluble in water and also dissolves in various other solvents. The cause is the intricate and resilient structure of nanocellulose, along with the amorphous component, which is eliminated during the hydrolysis process by acid. This component contributes to the elevated crystallinity. The increased utilization of nanocellulose enhances the stability of the emulsion and facilitates the integration of nanocellulose and water, aided by the employed solvent [10] [11]. Additionally, the heating temperature element contributes to this, as elevated temperatures facilitate the complete dissolution of nanocellulose in the sample due to its excellent structural stability.

### 3.2. Particle size

Table 2 illustrates that the sample particle size at each temperature diminishes with increasing nanocellulose content. The incorporation of nanocellulose concentration may inhibit high-temperature flocculation, hence preventing the reduction of nanocellulose particle size within the emulsion. The diminutive particle size will yield a stable emulsion system, preventing any separation. Temperature affects the characteristics of the resultant water, the interfacial film, and the emulsifier's solubility in both oil and water. Various particle sizes were also achieved

according to the formulation of nanocellulose concentration. At elevated concentrations, a reduction in particle size enhances the efficacy of nanocellulose in stabilizing the oil/water interface, attributable to its amphiphilic surface characteristics derived from the hydrophobic surfaces and hydrophilic edges of the cellulose chains. The diminutive particle size accelerates the occurrence of Brownian motion. The accelerated Brownian motion will inhibit sedimentation and yield a more transparent solution. The combination of high-temperature treatment and maximum nanocellulose concentration can diminish particle size, enhancing the oil-in-water emulsion's stability.

Table 2. Effect of temperature and nanocellulose concentration on particle size, particle charge, and viscosity in oil-in-water emulsions

Temp (°C)	Concentration (%)	Particle Size (nm)	Zeta Potential (mv)	Viscosity (cp)
60	0	657.66 ± 10.26 <sup>f</sup>	1.93 ± 1.51 <sup>a</sup>	0.85 ± 0.01 <sup>a</sup>
70		549.66 ± 8.50 <sup>e</sup>	1.86 ± 2.08 <sup>a</sup>	0.86 ± 0.01 <sup>b</sup>
80		199.23 ± 3.57 <sup>d</sup>	1.63 ± 1.02 <sup>a</sup>	0.87 ± 0.01 <sup>d</sup>
60	10	203.33 ± 3.34 <sup>d</sup>	3.06 ± 0.45 <sup>a</sup>	0.86 ± 0.01 <sup>c</sup>
70		171.40 ± 3.81 <sup>c</sup>	2.03 ± 0.80 <sup>a</sup>	0.87 ± 0.01 <sup>f</sup>
80		135.00 ± 3.11 <sup>b</sup>	1.66 ± 1.74 <sup>a</sup>	0.88 ± 0.01 <sup>h</sup>
60	20	175.90 ± 4.33 <sup>c</sup>	6.66 ± 0.20 <sup>b</sup>	0.87 ± 0.01 <sup>e</sup>
70		136.20 ± 4.33 <sup>b</sup>	2.93 ± 0.57 <sup>a</sup>	0.88 ± 0.01 <sup>g</sup>
80		102.30 ± 0.86 <sup>a</sup>	2.40 ± 0.45 <sup>a</sup>	0.88 ± 0.01 <sup>i</sup>

Note: The test was carried out in three repetitions. Different lowercase letters (a-f) on particle size, (a-b) on particle load and (a-i) on viscosity show significant differences ( $p < 0.05$ ).

### 3.3. Particle Charge

Table 2 illustrates the influence of heating temperature on the particle charge relative to nanocellulose content. The zeta potential is the potential difference between the layers on the surface that are closely associated with the electroneutrality in the formulation. This value helps ascertain the stability pattern and indicates the extent of attraction between neighboring particles with identical charges and scattered particles [12]. A zeta potential of ± 30 mV is considered adequate to guarantee the physical stability of the nanoemulsion. Zeta potential typically varies between +100 mV and -100 mV. Table 2 indicates a statistically significant difference in particle charge or zeta potential values ( $p < 0.05$ ) in samples with elevated nanocellulose concentration. The value of the particle charge will augment. The likelihood of flocculation or the aggregation of colloids from smaller to larger forms increases with a greater zeta potential value. Colloids exhibiting elevated zeta potential values are electrically stable, whereas those with diminished zeta potential are prone to coagulation or flocculation [13].

The emulsion's stability will enhance by incorporating suitable polymers in the dispersing phase and reducing the particle size of the dispersed phase. Cellulose fibers comprise crystalline and amorphous phases, with the crystalline phase being more prevalent than the amorphous phase. Applying heat can reactivate H<sup>+</sup> ions to interact with the amorphous phase of cellulose fibers; however, too high temperatures may lead to the carbonization of cellulose [14]. Using a moderate temperature combined with a brief heating duration and incorporating the greatest concentration of nanocellulose in this study effectively enhances the stability of oil-in-water emulsions since a high zeta potential inhibits flocculation.

### 3.4 Viscosity

Table 2 illustrates the impact of heating temperature on the viscosity of nanocellulose concentration. A high viscosity value may signify excellent emulsion stability. Emulsions undergoing flocculation exhibit increased viscosity due to the flocculation structure entrapping a continuous phase (water) [15]. Table 2 indicates a substantial variation in viscosity values ( $p < 0.05$ ). Viscosity values rise with elevated temperature and increased nanocellulose concentration. Agi [1] also revealed that hydrophobic bonding may be responsible for this phenomenon at elevated temperatures, as the solution binding system enhances tensile strength and facilitates the formation of cohesive solid bonds between particles [16].

Eichie & Amalime [17] assert that an increase in mixing temperature enhances the energy available, facilitating the emulsifier's formation of a film layer over the dispersed droplets, resulting in reduced droplet size and increased viscosity. Additionally, An appropriate viscosity is characterized by a greater viscosity value, which complicates particle movement, hence enhancing sample stability. Stokes' law posits that a reduction in particle size prolongs the creaming process, thereby necessitating the minimization of particle dimensions. Smaller particle sizes result in increased surface free energy, hence rendering the emulsion more unstable. These two statements indicate that a specific particle size must be attained. Elevating the mixing temperature supplies energy for the droplet to mobilize and fragment into a smaller size, facilitating the formation of an emulsifier to envelop the droplet. Elevated mixing temperatures can diminish the surface tension at the phase interface, allowing the oil phase to be completely dispersed inside the dispersing phase (water) [18]. Furthermore, employing an excessively high temperature will disrupt the bonds between the droplets and the emulsifier; for instance, the hydrogen bonds between polyoxyethylene sorbitan fatty acid ester (polysorbate/tween) and water molecules will be severed, resulting in diminished emulsion stability [19].

The reduction in emulsion stability is also affected by unsuitable material composition, inadequate quantity and selection of emulsifiers, freezing, and mechanical stress or vibration during production. In addition to temperature, the presence of a stabilizer, specifically Tween 20, influences the stability of emulsion viscosity. With the suitable Tween 20, the emulsifier can inhibit droplets from coalescing from the dispersed phase into the continuous outer phase, hence preventing the formation of bigger droplets. The reduction of droplets (dispersed phase) to the continuous phase can decrease viscosity. An increased concentration of the inner phase (smaller droplet size) results in a higher phase volume ratio, hence elevating the viscosity of the emulsion. Consequently, with respect to the viscosity parameters, increased concentrations of NCC and NFC, along with elevated temperatures, result in higher viscosity values. Increased viscosity can inhibit the migration of oil droplets, signifying enhanced emulsion stability.

### 3.5. Creaming Index

Figure 2 illustrates the impact of heating temperature on the concentration of nanocellulose on the creaming index. Creaming is a process of emulsion instability that initiates phase separation by the movement of droplets. The extent of phase separation in the emulsion system, represented by the creaming index, signifies a reduction in emulsion stability. As the concentration of the emulsifier increases, the strength of the resulting emulsion also increases until an ideal threshold is attained, beyond which a precipitate will form. Fat globules typically manifest on the emulsion's surface. This transpires because the density of fat is less than that of water. The emergence of fat on the emulsion's surface results in a clearly discernible cream

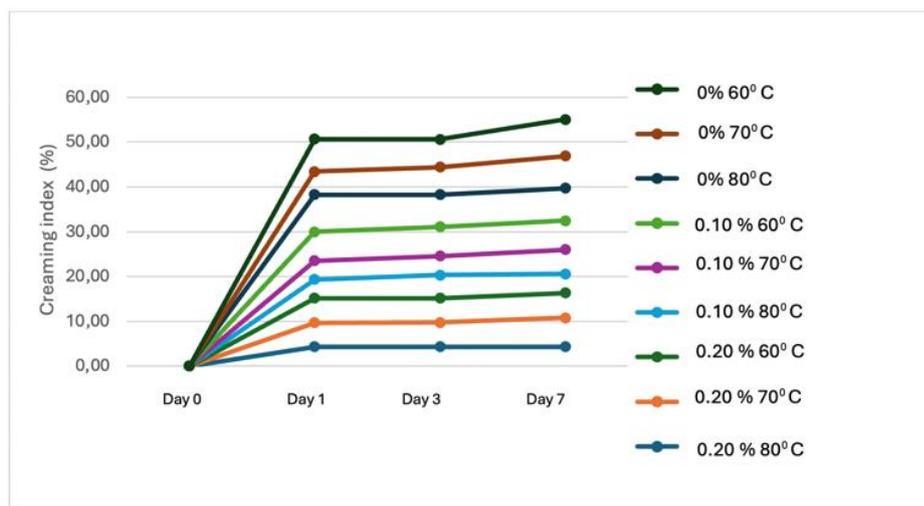


Figure 1. Effect of temperature and nanocellulose concentration on index creaming in oil-in water emulsions

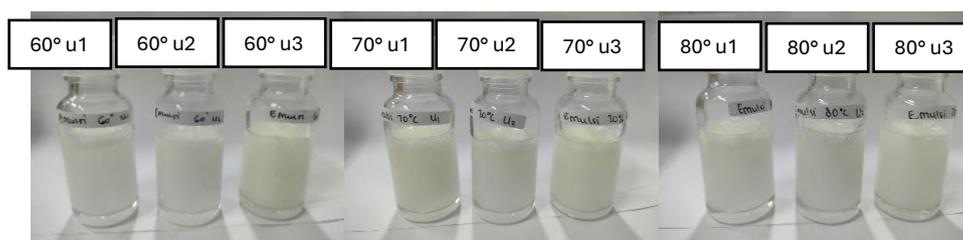


Figure 2. Creaming index at 60° C, 70° C, 80° C at concentration 0 % Day 0

On the first day of sample preparation, creaming had not commenced in samples with concentrations of 0%, 0.10%, and 0.20%. The creaming index in the samples was monitored until day 7, after which no additional creaming observations were conducted. The samples were stored for 30 days without any observations; they remained stable throughout this period. The stability of the emulsion phase is affected by the interaction of the emulsifier, Tween 20, in creating a firm and stable interfacial monolayer coating. The incorporation of nanocellulose into the emulsion network can enhance viscosity and reduce the rate of sedimentation and creaming caused by gravity forces. The relationship between nanocellulose and surfactants pertains to the attraction of non-ionic surfactants to the cellulose surface. This phenomenon can be ascribed to the hydrophobic contact between the surfactant's hydrophobic groups and the hydrophobic areas associated with the cellulose substance.

The graph of the creaming index rate indicates that at a concentration of 0% throughout all temperatures, the creaming index value is maximized. This may occur due to the sample concentration being 0% in the absence of nanocellulose addition. The creaming index value decreases with rising temperature and nanocellulose concentration. The incorporation of nanocellulose may lead to an increase in emulsion viscosity, hence impeding the ascent of oil droplets and diminishing the likelihood of creaming. The use of nanocellulose ensures prolonged shelf life and offers an aqueous dispersion with stability across a broad spectrum of pH and temperature conditions [28]. Consequently, augmenting the nanocellulose concentration in the emulsion and elevating the heating temperature can prolong its shelf life and preserve its stability.

#### 4. Conclusions

Incorporating NCC and NFC at 0.20% and 70°C reduced particle size and enhanced particle charge in o/w nanoemulsions, resulting in greater electrostatic repulsions. The viscosity rises with increased concentrations of the nanocellulose mixture and temperature. Consequently, the creaming index diminished, resulting in enhanced emulsion stability. The amalgamation of NCC and NFC, specifically at 0.20%, serves as a natural stabilizer to enhance the stability of o/w nanoemulsion at a temperature of 70°C.

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