

# Physical Properties and Stability of Emulsions as Affected by Native and Modified Yam Starches

Nor Hayati Ibrahim and Shamini Nair Achudan

**Abstract**—This study was conducted in order to determine the physical properties and stability of mayonnaise-like emulsions as affected by modified yam starches. Native yam starch was modified *via* pre-gelatinization and cross-linking phosphorylation procedures. The emulsions (50% oil dispersed phase) were prepared with 0.3% native potato, native yam, pre-gelatinized yam and cross-linking phosphorylation yam starches. The droplet size of surface weighted mean diameter was found to be significantly ( $p < 0.05$ ) lower in the sample with cross-linking phosphorylation yam starch as compared to other samples. Moreover, the viscosity of the sample with pre-gelatinized yam starch was observed to be higher than that of other samples. The phase separation stability was low in the freshly prepared and stored (45 days, 5°C) emulsions containing native yam starch. This study thus generally suggested that modified yam starches were more suitable (i.e. better physical properties and stability) to be used as stabilizers in a similar system i.e. light mayonnaises, rather than a native yam starch.

**Keywords**—Oil-in-water emulsions, low-fat mayonnaises, modified yam starches, droplet size distribution, viscosity.

## I. INTRODUCTION

YAMS are from the genus of *Dioscorea* and family of *Dioscoreaceae* and yam tubers are known to contain high amount of carbohydrate and protein. The most important carbohydrate constituent is starch and it has been reported that about 14.25% of starch can be isolated from *Dioscorea alata* [1]. However, there are a number of disadvantages present for the native yam starches in certain industrial applications. The problems occurred for native starch are the granules hydrate easily, swell rapidly, rupture, lose viscosity, and produce weak bodied, very stringy and cohesive pastes. Principally, the native yam starch will be improved to become better through the modification. Physical modification (e.g. pre-gelatinization) is known as the simultaneous action of several situations such as temperature, pressure, shear and moisture on the starch [20]. This procedure causes the starch granule being preserved or may be totally disorganized. On the other hand, chemical modification (e.g. cross-linking phosphorylation) is known as the insertion of functional groups into the starch

molecule where this will cause markedly altered physico-chemical properties [3].

Normally, thickening polysaccharides like xanthum gum, guar gum, carboxymethyl cellulose in combination with modified starch are being utilized in emulsion products like mayonnaise and salad dressing [4]. There are frequently used to stabilize the emulsion and to increase the viscosity of light mayonnaise [5]. In addition, modified starch was commonly utilized as a choice in food industry for food emulsion formulated with less amount of fat due to their special creamy texture and low cost [6]. This kind of polysaccharide has affected the vital character of products in term of their creamy mouthfeel, high yield stress for suspension ability, thickness and occasionally for long-term product stability. Moreover, the modified starches are the commonly agreeable substitute to gum Arabic for their usage in beverage emulsion stabilizer [7]. Although many studies have been carried out to modify yam starch but there was very limited research has been conducted to observe the performance of the modified yam starches in the emulsion-based food products such as mayonnaise. Thus, the objective of this study was to investigate the effects of native and modified yam starches on the physical properties and stability of the mayonnaise-like emulsions.

## II. MATERIALS AND METHODS

### A. Materials

Yam was purchased from Kelantan, Malaysia. Ingredients for mayonnaise-like emulsions such as egg, salt, sugar, vinegar and oil were obtained from local supermarket. Xanthan gum was purchased from Sigma (Sigma-Aldrich, St. Louis, MO).

### B. Modification of Starch

The isolation of starch from yam tubers was done based on a modified method described elsewhere [8]. The starch was then modified through pre-gelatinization and cross-linking phosphorylation procedures [9]. For pre-gelatinization, 100 g of yam starch was suspended in 333 ml of distilled water and heated until the temperature reached 80°C by slow manual stirring. Then, the pre-gelatinized starch was placed into stainless steel tray in form of thin film (1 to 2 mm) and dried in an oven at 40°C for 48 hours. For cross-linking phosphorylation 200 ml of 45% starch suspension was mixed with 10 g of sodium sulfate and 4 g of trisodium trimetaphosphate. The pH of suspension was adjusted to 9.5 by adding 10% aqueous hydrochloric acid or sodium hydroxide. The slurry was stirred for 1 hour at room temperature, and washed three times with distilled water. Later, the slurry was dried in an oven at 40°C to 12% moisture

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and heated in an oil bath for phosphorylation for 2 hours at 130°C. After cooling at room temperature, the starch cake was washed in distilled water and the starch was recovered by centrifugation at 1500 rpm for 10 minutes. Finally, the pH of suspension was adjusted to 6.5. The recovered starch was dried at 40°C in a vacuum oven. The starches obtained were ground to pass through a 250 µm screen and stored in glass jar at room temperature.

### C. Preparation of Mayonnaise-like Emulsions

The emulsions were prepared according to the method described previously [4] with a slight modification. The emulsions (100 g) were prepared in a lab-scale proportion using 50% soya bean oil, 10% water, 4% vinegar, 6% egg yolk and 30% polysaccharide dispersion. The final percentage of polysaccharide in total emulsion was 0.5% which contained 0.3% of xanthum gum and 0.2% starch. A premix was prepared with 10 g water, 4 g vinegar, 6 g egg yolk, and the oil (50 g) was incorporated dropwise. Homogenization was first carried out to emulsify the oil for 1 minute at speed 1. After that, 30 g of polysaccharide dispersion was slowly added and homogenization was continued for the next 4 minute at speed 2. Four types of emulsion were prepared, containing native potato starch (NPS), native yam starch (NYS), pre-gelatinized and cross-linked phosphorylated yam starches (PYS and CLPYS, respectively).

### D. Physical Properties and Stability Analyses

The droplet size distribution of the freshly prepared emulsions was determined by a laser diffraction method of a particle size analyzer (PSA) (Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK). Emulsions were diluted in distilled water (pH 3) to a droplet concentration of less than about 0.05 wt% (to eliminate multiple scattering effects), and gently homogenized (to increase the homogeneity) using vortex prior to measurement. Drops of emulsion were introduced into the sample presentation unit until the concentration reached the optimum one, indicated by the instrument [4].

The viscosity of the emulsions was determined by rheomat (RM180, Rheometric Scientific Inc., New Jersey, USA). The measurement involved the freshly prepared emulsion. A measure of viscosity (Pa.s) was given for a shear rate of 200 Pa s<sup>-1</sup> for all samples using probe 3. The viscosity measurement was taken at 1 minute intervals for 6 readings [10].

A method modified from a previous study [11] was used to determine the turbidity. It was monitored by measuring the absorbance (loss rate of turbidity) at 500 nm by means of a UV-visible spectrophotometer (UV-1700, Shimadzu corp., Kyoto, Japan). The emulsion was diluted to 0.25% (w/w) in a 10% sugar solution and was stored in 1 L blue cap bottles at room temperature before the absorbance reading. To evaluate the stability towards phase separation, the freshly prepared emulsions were accurately weighted (10 g) into a 15 ml Teflon centrifuge tube and then centrifuged using a large capacity variable speed centrifuge (Hettich Universal 32, Hettich Zentrifugen Inc., Germany) at room temperature. The total height of each emulsion in the centrifuge tube was then measured. The experiment was also carried out for the stored

emulsions that already placed in the centrifuge tubes for 45 days. The calculation of percentage of separated layers for each emulsion was as follows [4]:

$$\% \text{ layer} = (\text{the height of the separated layer} / \text{the total height of the emulsion}) \times 100\%.$$

### E. Experimental Design and Statistical Analysis

The independent variables (treatments) of this experiment were different types of starch while the dependent variables were physical properties (viscosity and droplet sizes) and stabilities (centrifugation assay and turbidity) of the emulsion. Each emulsion was prepared in three independent replications based on a completely randomized design. The statistical analysis was carried out by means MINITAB (Release 14.0) statistical software package using an  $\alpha = 0.05$  confidence level. A one-way analysis of variance (ANOVA) was applied to determine significant differences between the mean followed by Tukey's HSD for comparison among groups. In the case of viscosity and turbidity, data were first analyzed using two-way ANOVA to find significant interaction effect between treatments and time. If there were no significant differences in interaction effect, one-way ANOVA with Tukey's HSD were then proceeded to find significant effect of both variables.

## III. RESULTS AND DISCUSSION

### A. Droplet Size Distribution

For emulsion droplet size, the diameter explaining surface area ( $d_{3,2}$ ) are important when the study of a catalyst or the amount of surfactants on the droplets' surface are of interest whereas the diameter to the mass or volume ( $d_{4,3}$ ) of the dispersed oil droplets are referred when total amount of oil in the dispersed phase is significant (i.e. 50% in the present case). According to McClemants (2005),  $d_{4,3}$  is more sensitive to the existence of large particles in an emulsion compared to  $d_{3,2}$ . Thus,  $d_{4,3}$  more sensitive to the phenomena of flocculation. This might explain the higher value of  $d_{4,3}$  compared to  $d_{3,2}$  since all emulsions showed large droplets. All emulsions exhibited a typical bimodal droplet size distribution with a pronounced shoulder reflecting a very small group of droplet with the largest diameter (Fig. 1). Based on the analysis of one-way ANOVA, it was found that there were significant differences among the samples ( $p < 0.05$ ) for  $d_{3,2}$  with emulsion containing CLPYS showed the smallest droplet size, followed by those with NYS, PYS and NPS. On the other hand, it was found that there were no significant differences among the samples for  $d_{4,3}$  (Table I).

Normally, native potato and native yam starches have hydrogen bonds which are hydrophilic. During the physical modification (pre-gelatinization), some of the hydrogen will be broken down which resulted in the decrease of the hydrophilic behavior of pre-gelatinized yam starch. Pre-gelatinization process was based on the rearrangement of intra- and intermolecular hydrogen bonding between the water and starch molecules [12]. These rearrangements will result in the collapse or disruption of molecular orders within the starch granule and cause irreversible changes in the starch properties. The starch granule might be preserved or totally disorganized [2]. As a result, this could decrease the ability of the emulsion

containing PYS which might not enough to cover the oil droplets and form sufficiently adsorption layer. This reason might explain the larger droplet size in this emulsion. On the other hand, emulsion containing CLPYS shows the smallest droplet size among the other emulsions. Cross-linking normally can strengthen the hydrogen bonds in the granule where the chemical bonds function as bridges between the starch molecules by bonding covalently to inter and intramolecular bridges between starch polymers [13]. This can result in more hydrophilic behavior of the starch which easily emulsified the droplets and made them become smaller. As a result, the starch might be enough to cover the oil droplets and form sufficiently adsorption layer.

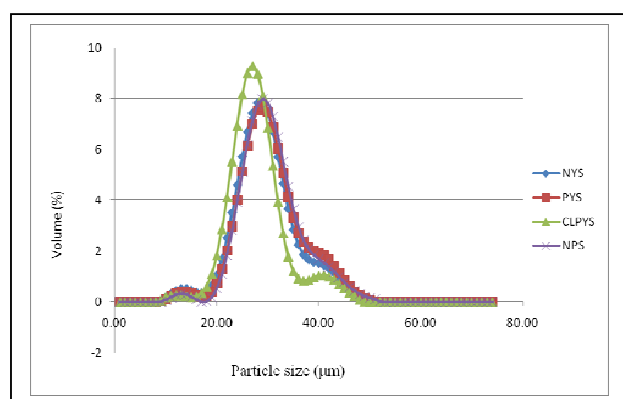


Fig.1 Droplet size of the freshly prepared emulsions with different treatment. Each point represents two replicates data. NYS, Native Yam starch; PYS, Pre-gelatinized Yam Starch; CLPYS, Cross-linking Phosphate Yam Starch; NPS, Native Potato Starch

TABLE I  
DROPLET SIZES OF THE FRESHLY PREPARED EMULSIONS

Emulsions	$d_{3,2}$ (µm)	$d_{4,2}$ (µm)
Native yam starch	14.239 <sup>ab</sup>	31.529 <sup>a</sup>
Pre-gelatinized yam starch	16.076 <sup>a</sup>	35.136 <sup>a</sup>
Cross-linking phosphate yam starch	13.059 <sup>b</sup>	29.815 <sup>a</sup>
Native potato starch	16.162 <sup>a</sup>	37.947 <sup>a</sup>
Standard deviation range	0.546 – 1.153	1.260 – 5.189

Data are reported in mean from 3 replications. <sup>a-b</sup> Mean with same small letter within the same column are not significantly different.

#### B. Viscosity

There are two independent variables involved in this analysis which were types of starch and time and the interaction of these two variables can affect the viscosity of the emulsion as shown in Fig. 2. The results showed that the emulsion viscosity was strongly depended on the types of starch and modified starch used in the continuous phase (Table II). The viscosity was observed to be the highest in the emulsion incorporated with NPS followed by PYS, then NYS and lastly the viscosity was the lowest in the emulsion containing CLPYS. There were significant differences among the sample ( $p < 0.05$ ) and the trend of changes in the viscosity were similar at each time. The viscosity of emulsion incorporated with NPS was significantly different ( $p < 0.05$ ) from that of NYS and CLPS but not significantly different from that of emulsion with PYS.

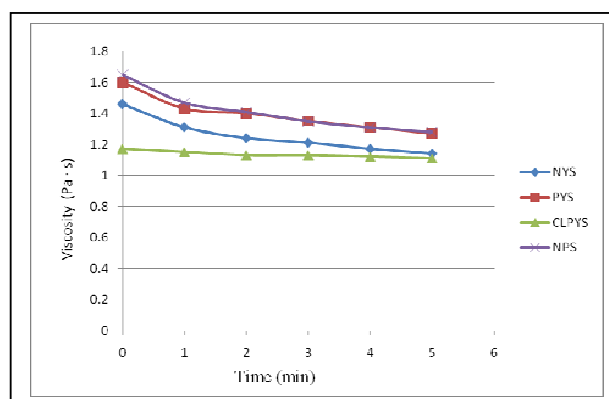


Fig. 2 Viscosity of the freshly prepared emulsions. Data are reported in mean from 3 replications. See Fig.1 for abbreviation of the samples

TABLE II  
VISCOSITY OF THE FRESHLY PREPARED EMULSIONS

Emulsions	Viscosity(Pa s <sup>-1</sup> )					
	0 min	1 min	2 min	3 min	4 min	5 min
Native yam starch	1.46 <sup>ab</sup>	1.31 <sup>ab</sup>	1.24 <sup>ab</sup>	1.21 <sup>b</sup>	1.17 <sup>ab</sup>	1.14 <sup>ab</sup>
Pre-gelatinized yam starch	1.60 <sup>a</sup>	1.43 <sup>a</sup>	1.40 <sup>a</sup>	1.35 <sup>a</sup>	1.31 <sup>a</sup>	1.27 <sup>a</sup>
Cross-linking phosphate yam starch	1.17 <sup>b</sup>	1.15 <sup>b</sup>	1.13 <sup>b</sup>	1.13 <sup>b</sup>	1.12 <sup>b</sup>	1.11 <sup>b</sup>
Native potato starch	1.65 <sup>a</sup>	1.47 <sup>a</sup>	1.41 <sup>a</sup>	1.35 <sup>a</sup>	1.31 <sup>a</sup>	1.28 <sup>a</sup>

Data are reported in mean from 3 replications. <sup>a-b</sup> Means with same small letter within the same column are not significantly different. Standard deviation range = 0.02 – 0.16 Pa s<sup>-1</sup>.

Generally, potato starch is known for its high viscosity and forming pastes and gels with long consistency. The apparent amylose content has been reported to be higher in potato starch (37.8%) than in yam starch (29.2%) [14]. Moreover, the viscosity of the emulsion containing NYS was found to be lower than those containing PYS. In the contrary, the viscosity of the emulsion containing native yam starch was observed to be higher than the cross-linking phosphate yam starch. Normally, chemical modification involved the incorporation of chemically bonded bridges and this as a result can retain the granule integrity in the presence of water and also retain the condition promotes rupture of the hydrogen bonds responsible for the granule integrity [15].

Furthermore, the droplet size also can influence the viscosity of the emulsion [16]. Normally, the viscosity is higher in emulsion with smaller average droplet diameter compared to the ones with larger average droplets diameter. This might be due to the decrease in the mean separation distance which causes the increase in the hydrodynamic interaction. In addition, the decrease in droplet size could also be due to the aggregation or flocculation of droplets. When the droplet size and mean separation distance between droplets decrease, this will cause the increase in the chances of aggregation/flocculation due to *Van Der Waal's* attraction. Although the emulsion with CLPYS had the largest droplet size but the viscosity of this mayonnaise was observed to be

the lowest. Moreover, the emulsion containing NPS had the highest viscosity although this emulsion showed the largest droplet size. According to previous researcher [16], the effect of droplets size on the viscosity of oil-in-water becomes significant when the dispersed phase concentration has a high concentration (or diluted emulsion). Only the concentrated oil-in-water emulsion with small droplets results in an increase of viscosity. This was previously observed for xanthan gum-based emulsion with a higher droplet size which showed higher viscosity and yield stress [4]. It was thus suggested that the rheology of the concentrated emulsion was more influenced by their continuous phase that consist of polysaccharide rather than its droplet size. In the present case, the viscosity of the continuous phase with NPS itself contributed to the viscosity of the emulsion containing it regardless to the droplet size.

As discussed before, all emulsions with different treatments showed decreases in viscosity with time. This indicates that all emulsions showed a thixotropic behaviour exhibiting decreases in apparent viscosity with time although the shear rate is constant [18]. Emulsion with this behavior usually contains droplets that are aggregated by weak forces. Shearing of the material makes the aggregated particles to gradually deformed and disrupted. This cause decline in the resistance to flow and resulted in decrease of apparent viscosity over time. The high thixotropic behaviour results in greater breakdown during the stirring process [16]. Therefore, the thixotropic behaviour was high in emulsion containing NPS, followed by PYS, then NYS and lastly in CLPYS (Fig. 2). Usually, cross-linked starches are resistant to high temperature and a prolong shear [13]. Cross-linking normally can strengthen the hydrogen bonds in the granule where the chemical bonds function as bridges between the starch molecules by bonding covalently to inter and intramolecular bridges between starch polymers [13, 17]. This reason might explain a lower time-dependent viscosity observed for the emulsion with cross-linking phosphate yam starch compared to other emulsion.

The absence of covalent bond in emulsion containing NYS and NPS might explain the high time-dependent viscosity in their respective emulsions since this starch is the native starches. Normally, pre-gelatinization is the physical modification of starch with simultaneous action of several situations such as temperature, pressure, shear and moisture on the starch [2]. There is no addition of the covalent bond in the pre-gelatinized starch. Therefore, the shear rate also high in the emulsion containing pre-gelatinized yam starch.

### C. Physical Stability

The turbidity of the freshly prepared emulsions is shown in Table III. Normally, a higher turbidity value indicates a more stable emulsion system. There were significant effects ( $p < 0.05$ ) of starches on the turbidity of the emulsion. The turbidity was observed to be the lowest in the emulsion incorporated with NPS followed by CLPYS, then PYS and lastly the turbidity was the highest in the emulsion containing NYS.

TABLE III  
TURBIDITY OF THE FRESHLY PREPARED EMULSIONS

Emulsions	Turbidity (nm)					
	0 sec	10 sec	20 sec	30 sec	40 sec	50 sec
Native yam starch	0.33 <sup>a</sup>	0.33 <sup>a</sup>	0.32 <sup>a</sup>	0.32 <sup>a</sup>	0.32 <sup>a</sup>	0.32 <sup>a</sup>
Pre-gelatinized yam starch	0.27 <sup>ab</sup>	0.27 <sup>ab</sup>	0.27 <sup>ab</sup>	0.26 <sup>ab</sup>	0.26 <sup>ab</sup>	0.26 <sup>ab</sup>
Cross-linking phosphate yam starch	0.26 <sup>b</sup>	0.26 <sup>b</sup>	0.26 <sup>b</sup>	0.25 <sup>b</sup>	0.25 <sup>b</sup>	0.25 <sup>b</sup>
Native potato starch	0.240 <sup>b</sup>	0.237 <sup>b</sup>	0.232 <sup>b</sup>	0.229 <sup>b</sup>	0.226 <sup>b</sup>	0.223 <sup>b</sup>

Data are reported in mean from 3 replications. <sup>a,b</sup> Means with same small letter within the same column are not significantly different. Standard deviation range = 0.01 – 0.04 nm.

The turbidity of the prepared emulsions as a function time is depicted in Fig. 3. Usually the emulsion stability is influenced by the magnitude of interaction and forces (as barrier energy) between emulsion droplets [11] which is related to creaming properties of the all emulsions. This is because creaming involves the movement of the droplet to upward whereas turbidity also involves the movement of the particle to upward. The higher creaming rate, coalescence rate and the probability of flocculation can be caused by the larger mean droplet size. On the contrary, greater emulsion stability with respect to coalescence can be produced by the smaller droplets.

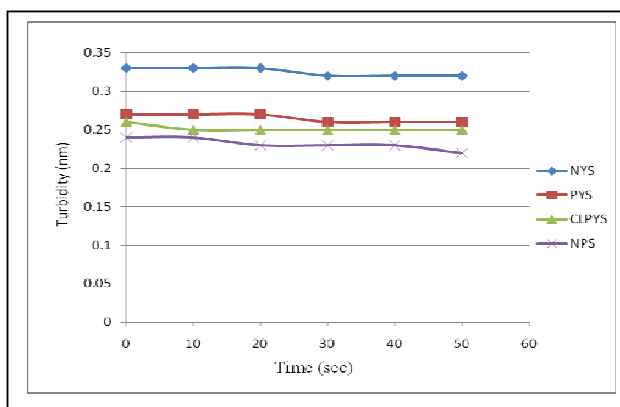


Fig. 3 Turbidity of the freshly prepared emulsions. Data are reported in mean from 3 replications. See Fig.1 for abbreviation of the samples

Normally, the large droplets size will result in lower turbidity value whereas the smaller droplets size will result in high turbidity value. This is because emulsions with large droplets have more tendencies to migrate quickly to the middle and upper part of the cuvette which resulted in lower turbidity reading. The turbidity was observed to be lower in emulsion containing NPS with large droplet size than emulsion CLPYS. Moreover, the emulsion containing PYS had lower turbidity than the NYS although the larger droplet was obtained from the former emulsion. In a different way, small droplets could form large flocs whereby the flocs also tend to move upwards which is true for concentrated emulsion [18] as found in the present case. This could explain a lower turbidity value of CLPYS emulsion with smaller droplet size and larger turbidity of PYS emulsion with large droplets size.

The phase separation of freshly prepared and stored emulsions by using centrifugation forces is shown in Table IV. All emulsions formed 2 layers after centrifugation. The upper layer or the cream phase of the emulsion contained an emulsion phase whereas the bottom layer contained an aqueous phase. There were significant differences among the samples ( $p < 0.05$ ) in term of phase separation stability. From this study, it was found that all emulsions showed syneresis regardless of the type of starch content. It was observed that NYS and PYS emulsions were significantly stable towards syneresis as compared to others. These results seemed to be parallel with the turbidity results.

TABLE IV  
PHASE SEPARATION OF THE FRESHLY PREPARED  
AND STORED EMULSIONS

Emulsions	Layer of Aqueous phase (% of total emulsion height)	
	Freshly prepared	Stored (45 days)
Native yam starch	26.99 <sup>b</sup>	32.70 <sup>b</sup>
Pre-gelatinized yam starch	28.57 <sup>b</sup>	33.65 <sup>b</sup>
Cross-linking phosphorylated yam starch	33.33 <sup>a</sup>	33.97 <sup>b</sup>
Native potato starch	33.97 <sup>a</sup>	42.86 <sup>a</sup>
Standard deviation range	0.548 – 0.955	0.554 – 1.905

Data are reported in mean from 3 replications. <sup>a,b</sup> Mean with same small letter within the same column are not significantly different.

The extent of syneresis in the emulsions could be attributed to a water holding capacity of starch in the aqueous continuous phase. The difference in the water holding capacity among starches can be due to the variation in the degree of the engagement of hydroxyl groups to produce hydrogen and covalent bonds between starch chains [19]. The water holding capacity of native potato starch seemed to be the lowest among other starches, resulting in the most unstable emulsion towards phase separation. In this study, although all the emulsion have stored for 45 days, it was found that there is no an oiling off occurred. This suggests that there is no severe droplet coalescence during storage.

The physical properties and stability of the emulsions was highly influenced by the type of modified starch present in the aqueous continuous phase. Of two modifications used, pre-gelatinization is more suitable method to modify the yam starch for utilization in mayonnaise-like emulsions. The starch was found to be significantly increased the viscosity and physical stability of the prepared emulsion. Thus, modified yam starch has the greater ability to be used as stabilizer to replace the currently used native potato starch.

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