DEVELOPMENT OF YANANG (*TILIACORA TRIANDRA*) POWDER USING FREEZE DRYING IN USE FOR SHERBET.

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ABSTRACT

Yanang powder is a functional ingredient that can be used for enhancing the nutritional value of frozen dessert products. In this study the effect of fortification with different concentrations of freeze-dried Yanang powder on physicochemical properties, functionality, microbial risk and sensory quality of sherbet with 10%, 20% and 30% of Yanang powder was investigated. Total solids content and pH values of the samples were found to increase with increased level of Yanang powder addition. Average overrun of sherbet products was decreased when the concentration of freeze-dried Yanang powder increased but the sherbet with fresh Yanang extract had overrun no significant difference from a control. Hardness and viscosity increased but melting rate decreased when the concentration of freeze-dried Yanang powder increased. The highest amount of Yanang powder added into sherbet presented the highest TPC value corresponding with higher DPPH and FRAP values which made the sherbet a good promising antioxidant product. The addition of Yanang powder affected appearance/color, texture, flavor, foreign taste and overall acceptability of sherbet samples. Based on sensory evaluation, the best amount of freeze-dried Yanang powder fortified in sherbet was at 10% compared with fresh Yanang extract at the same level (10%).

Keywords: Antioxidant activity, Freeze-dried powder, Sherbet, Yanang

INTRODUCTION

Consumers' trends nowadays prefer foods prepared with low in fat, more natural antioxidants, and free of synthetic additives. Yanang (*Tiliacora triandra*) is a species of flowering plant widespread in the northeast of Thailand. Its leaves contain polyphenols, flavonoids, alkaloids, high levels of beta-carotene and minerals, including fiber, calcium, iron, vitamin-A, vitamin-C, and phosphorus [1]. The phytochemicals extracted from this plant have been used as herbal medicines for fever relief, anti-pyretic, detoxication agent, anti-inflammation, anticancer, antibacterial, and immune modulator, especially antioxidant capacity [2]. However, Yanang extracts have not becoming popular as a food ingredient due to its greenish smell. To overcome its limitation, the encapsulation of phytochemicals extracted and transform into dried form, which is a great process for the flavoring and food additive industries, could be an alternative way to fortify Yanang in food products [3]. This technique encapsulates bioactive compounds in liquid form in a carrier matrix to obtain a dry powder, which the advantages not only ease to handle in the solid state, but also protect encapsulated load against degradative reactions and loss of functionality during food processing, storage and consumption.

The powerful encapsulating agent is a hydrolyzed maltodextrin widely used as a wall material in the microencapsulation of food ingredients. Its use offers advantages such as relatively low cost, neutral aroma and taste, low viscosity at high solid concentrations and good protection against oxidation [4]. Different processes are available to prepare encapsulating powder. Freeze drying is one of the most applicable processes for drying thermosensitive substances that unstable in aqueous solutions. The drying process in which the sample is frozen and then dried by direct sublimation of the ice under reduced pressure [5]. The obtained powder is generally considered low bulk density, high porosity as well as good aroma and taste retention [6].

Due to the lack of information related to the application of freeze-dried Yanang powder fortified in foods, the aims of this research were to produce Yanang powder using a freeze dryer to be used as an antioxidant ingredient. Maltodextrin was added as a wall material. Then, the freeze-dried Yanang powder was added into sherbet formulation and the effect of Yanang powder on physicochemical property, microbiological risk, and sensory acceptance in addition to antioxidant activity of the Yanang sherbet was evaluated. Similar to ice cream, sherbet is usually processed with a higher quantity of fruit pulp and lower quantity of milk considered as reduced-fat ice cream exhibited some interesting regional characteristics, but poor in dietary fibers and some of the natural antioxidants [7]. Thus, the enrichment of sherbet with Yanang powder could be an effective way to enhance the nutritional and structural characteristics of frozen dessert.

MATERIALS AND METHODS

1. Chemical and reagents

Maltodextrin (Dextrose Equivalent, DE=10), acetic acid (ACS grade), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Trolox (6-hydroxy-2,5,7,8-tetramethylch roman-2-carboxylic acid), gallic acid, quercetin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Folin-Ciocalteu reagent and plate count agar (PCA) were obtained from Merck (Darmstadt, Germany). All chemicals used were of analytical research grade.

2. Preparation of Yanang juice

Thai herbal plant, Yanang (*Tiliacora triandra*) was freshly harvested from the orchards in Singburi province, Thailand. The leaves were cleaned with water to remove dust and dried using a tray dryer at 60 °C for 3 h. The Yanang juice was extracted with water using a ratio of 1:2 (w/v) and the obtained extract was stored before subjected to freeze drying.

3. Freeze drying of Yanang powder

Freeze drying was performed in a laboratory scale freeze dyer (Christ Alpha, Germany). The obtained Yanang extract was mixed with maltodextrin at a rate of 15% of the total solid content using a homogenizer (T10 Ultra-turrax, IKA, Germany) at 500 rpm for 5 min and then subjected to freezing in static air in a freezer at a temperature of -80 °C until processing time. Then, the frozen sample was taken to a freeze dryer at -50 °C for 72 h under pressure below 0.110 mbar. The dried Yanang product was then milled using a grinder and stored in desiccator until use.

4. Moisture content, a_w, % yield of Yanang powder and color

The moisture content was gravimetrically determined by the oven method at 105.2 °C in a hot air oven for 24 h [8]. The water activity (a_w) of Yanang powder was determined using water activity meter (Model: Decagon AquaLab LITE, Decagon Devices, Inc., USA). The yield of Yanang powder was then calculated using equation below:

$$\text{Yield (\%)} = \frac{\text{Weight of powder (g)}}{\text{Solid content of Yanang juice (g)} + \text{Weight of maltodextrin added (g)}} \times 100$$

The color of the powder (L^*, $a^*$and b^* values) was measured using colorimeter (Minolta CR 400, Japan). The measurement was carried out in triplicate for each experiment and the average value was reported.

5. Analytical antioxidant capacity of Yanang powder

Total soluble phenolic constituents of the Yanang powder was determined using Folin-Ciocalteu reagent with gallic acid as standard adopted from [9] with slight modification. Briefly, 20 μl of the solution sample was thoroughly mixed with 1.58 ml of water and 100 μl Folin-Ciocalteu reagent were added and incubated for 5 min at room temperature. Following incubation, 300 μl of Na_2CO_3 (2% w/v), solution was added into the mixture and vortexed. The mixture was incubated in dark for 2 h and the absorbance was measured at 765 nm using a UV-Vis spectrophotometer (GENESYS 10 UV, Thermo Scientific, USA). The total phenolic content (TPC) was expressed as milligram of gallic acid equivalents (GAE) per 1g of dry weight.

Free radical scavenging activity was also determined using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the procedure described by Brand-Williams, Cuvelier, and Berset (1995) with slight modification [10]. Briefly, 50 μl of standard samples were added to 1.9 μl DPPH methanolic solution. The reaction mixture was kept in the dark at room temperature for 30 min and the absorbance was measured at 515 nm. Trolox was used as a standard control. DPPH free radical capacity was calculated from a calibration curve and result was expressed as milligram of Trolox equivalents per 1g of dry weight.

The ferric reducing power of the Yanang powder was determined by using the potassium ferricyanide–ferric chloride method [11]. Briefly, 1 mL of sample was mixed with 2.5 mL of 0.2M phosphate buffer (pH 6.6) and 2.5 mL 1% potassium ferricyanide and the solution was incubated at 50 °C for 20 minutes. After that, 2.5 mL of 10% trichloroacetic acid was added to the incubated mixture. Then, 2.5 mL of distilled water and 0.5 mL 1% iron (III) chloride were added into 2.5 mL of the mixture and was incubated for 30 minutes. The absorbance was measured at 700 nm and the ferric reducing antioxidant power (FRAP) was expressed as milligram of gallic acids equivalents (GAE) per 1g of dry weight.

6. Preparation of Yanang sherbet

Yanang sherbet was devised based on the formular for plain sherbet mix recipe given by Whelan, Vega, Kerry, and Goff (2007) and Goff (2018) [12, 13]. Corn syrup solids, gelatin, non-fat dry milk powder and lime juice were obtained from a local supermarket. Sherbet base mix was warmed to dissolve corn solids and gelatin. Mix was aged overnight and then frozen with a home-style freezer (Richmond Cedar Works Mfg. Co., Danville, VA). Each product was packed in paperboard containers and stored in a freezer.

7. Physiochemical analyses of Yanang sherbet

Physicochemical properties of Yanang sherbet were evaluated according to Muse and Hartel (2004) method [14]. To measure the pH and total soluble solids (°Brix) contents of the samples, a pH meter (Ohaus Starter 3100, USA) and a digital refractometer (Atago PAL1, Japan) were used at 20 °C, respectively. The overrun of each product was calculated by measuring the weight of a volume of mix before and after freezing.

The color of the Yanang sherbet (L^*, a^* and b^* values) was measured using colorimeter (Minolta CR 400, Japan) as described in section Analytical antioxidant capacity of Yanang powder. The measurement was carried out in triplicate for each experiment and the average value was reported.

To evaluate the textural properties, a texture analyzer (LLOYD, TA plus Ametek, UK) was used to determine the hardness of the Yanang sherbet samples stored in containers at -

18 °C for 24 h. The analysis was carried out at 25 °C. For each sample, three measurements were carried out using a cylindrical probe (1 mm diameter) attached to a 1kN load cell. The penetration depth at the geometrical center of the samples was 10 mm and the penetration speed was set at 2 mm/s. The hardness expressed as the peak pressure force (g) during penetration.

To evaluate the viscosity of Yanang sherbet using Brookfield Viscometer (Brookfiled Viscometer, DVII, USA). Yanang sherbet samples were incubated at 8 °C and viscosity was measured using spindle no.2 to take torque measurements at 100 rpm. The viscosity was recorded as centipoise (cP).

To determine the meltdown of ice cream, 80.0±2 g of sample was put on a wire mesh attached to a graduated cylinder and maintained under a controlled temperature chamber at 25 °C and environment of constant relative humidity (50±1%). The dripped volume was measured at a 10 minute intervals for a total of 45 min. The first drop time was measured as the volume drip per minute. The data recorded was used to determine the melting rate (mL/min).

To evaluate the antioxidant capacity of fortified Yanang powder sherbet, total phenolic content was analyzed using Folin-Ciocalteu method with slightly modification as described in section 2.5. Free radical-scavenging activity was measured using the DPPH method with slightly modification as described in section analytical antioxidant capacity of Yanang powder.

8. Sensory analysis

Sensory evaluation was performed applying the 9-points hedonic scale test for appearance/color, texture, smoothness, melting resistance, flavor, foreign taste (1=undesired, 9=desired) and general acceptability (1=dislike, 9=like). All samples were identified with a random 3-digit code, and the serving order was also randomized. The panelists were 30 students in the Faculty of of Science and Technology at Suan Sunandha Rajabhat University (10 males and 20 females: age 20–22 years) and they were encouraged to write additional comments on the evaluation worksheet.

9. Microbiological analysis

Total viable count (TVC) was determined after 1 month on duplicate sets of petri dishes containing PCA by pour plate technique [15]. The plates were incubated at 37 °C for 24 h (AOAC, 2000). All results were expressed as CFU/mL sherbet.

10. Statistical analyses

ANOVA analysis of triplicate results was made using SAS 8.2 program (SAS, 1999) to compare the effects of pressure levels and treatment time. Comparisons among the means were made using Tukey *post-hoc* test ($p < .05$).

RESULTS AND DISCUSSION

1. Yanang powder property

In food industry, yield is an important economic consideration. The higher the yield obtaining, the better efficient the process. It is hypothesized that there is no or insignificant product loss during freeze drying and maltodextrin was added to increase the glass transition temperature of the powder. The result showed that the yield of freeze drying is satisfied.

Table 1. Physiochemical and antioxidant properties of freeze dried Yanang powder

Properties	Freeze-dried Yanang powder
Yield (%)	96.05±2.78
Moisture content (%)	2.81±0.14
Water activity (a_w)	0.09±0.01
Color, L^* a^* b^*	65.49±0.62 -2.79±0.01 15.21±0.14
TPC (mg GAE/1g)	0.94±0.04
DPPH (mg Trolox equivalent/1g)	0.29±0.02
FRAP (mg GAE/1g)	0.34±0.03

Results are presented as mean ± SD (n = 3).

As shown in Table 1, freeze-dried Yanang powder has low moisture content of 2.81%. Ibarz and Barbosa-Cánovas (2002) stated that freeze-dried product with moisture content lower than 2% can be obtained due to drying time [16]. It was suggested that the freeze drying time in this research can be prolonged in order to obtain freeze-dried Yanang powder of even lower moisture content. Apart from moisture content, water activity (a_w) is another important parameter to determine and predict shelf stability of the dried food product as it will affect the safety of food. The water activity of freeze dried Yanang powder was reported as 0.09 (Table 1). Since the water activity was lower than 0.50, therefore the powder would have lower risk of spoilage due to microbial reactions after fortified into food products.

Color parameters (L^*, a^* and b^*) of the freeze-dried Yanang powder were shown in Table 1. Freeze drying process uses low temperature which is expected to better preserve the color of the obtained powder. The antioxidant activity can be examined by different mechanisms, such as the prevention of peroxides, free radical scavenging, reducing capacity and binding of transition-metal ion catalysts [17]. A free radical DPPH accepts an electron or hydrogen radical to become a stable diamagnetic molecule [10]. The reduction capability of DPPH radical was done by the decrease in absorbance with antioxidants. The reducing power of FRAP assay serves as a significant indicator of its antioxidant activity. The reduction of the Fe^{3+}/ferricyanide complex to form the ferrous (Fe^{2+}) was monitored due to antioxidants [18].

Total phenolic content (TPC) of freeze-dried Yanang powder was 0.94 mg GAE/g (Table 1). It was close to the value proposed by Singthong et al. (2014) [1]. The TPC of fresh Yanang extracts by water was 0.98 mg GAE/g dry weight. Trolox equivalent of DPPH and gallic acid equivalent of FRAP were 0.29 mg and 0.34 mg per gram, respectively. Thus, natural pigments in Yanang are heat sensitive, the destruction of total phenolic content was protected due to low temperature utilized in freeze drying process.

2. Sherbet formulation

Control sherbet was prepared by using the formulation developed by Whelan et al. (2007) and Goff (2018) and the formulations were shown in Table 2 [12, 13]. Freeze dried Yanang powder was fortified to sherbet at a ratio of 0%, 10%, 20%, and 30% compared with sherbet added Yanang extract solution as the same weight of freeze dried Yanang powder of 10% (Table 2). The total solid of Yanang extract was 5%, so the extract solution substituted with water in sherbet formula was 10g and for freeze dried powder was 0.5 g, 1.0 g, and 1.5 g for 10%, 20%, and 30% respectively as seen in Table 2.

Table 2. Formulations of Yanang sherbet products

Ingredients (g)	**Freeze-dried Yanang powder**				**Yanang juice 10%**
	0%	**10%**	**20%**	**30%**	
Cream	7	7	7	7	7
Non-fat milk powder	3.5	3.5	3.5	3.5	3.5
Sugar	28	28	28	28	28
Corn syrup	0.1	0.1	0.1	0.1	0.1
Gelatin	0.2	0.2	0.2	0.2	0.2
Water	58.1	57.6	57.1	56.6	48.1
Citric acid	0.3	0.3	0.3	0.3	0.3
Lime juice	2.8	2.8	2.8	2.8	2.8
Freeze-dried Yanang powder	0	0.5	1.0	1.5	0
Yanang juice	0	0	0	0	10

3. Physical property of Yanang sherbet

Increased Yanang powder concentration increased pH values of sherbet. Also, the increasing concentration of freeze-dried Yanang powder significantly increased total soluble solid of sherbet (Table 3). The total soluble solids of the sherbet are contributed by the addition of Yanang powder that also gives essential physical property change such as texture. Several studies found that ice cream with lower total soluble solids may have proportionately more water to freeze, thus contributing to more ice crystal formation [19], influencing the texture and body of sherbet. Thus, texture differences will directly influence the consumers' decisions on the sherbets' quality.

The color of sherbet without adding Yanang powder has L* of 87.35, after added the powder, the values trended to decrease and the most concentration showed the lowest L* values. The same results found for a* and b*. The control had greenness and bright, when added more Yanang powder, the sherbet had darker, compared to sherbet fortified with fresh Yanang extract, which show brighter green.

It was found that the average overrun of sherbet, based on different freeze-dried Yanang powder concentrations, were decreased from 54.62% to 25.59% when the concentration of freeze-dried Yanang powder increased (0% to 30%); however, the sherbet with fresh Yanang juice had overrun no significant difference from the control. This value is lower than the general literature value of 80-100% of creamy ice-cream [20]. A possible reason for getting a lower overrun value is due to the sherbet itself use low fat content. When adding more solid contents, the overrun values were decreased.

Table 3. Physical properties and pH of Yanang sherbet products

Analyses	**Freeze-dried Yanang powder**				**Yanang juice 10%**
	0%	**10%**	**20%**	**30%**	
pH	3.13 [a]	3.35 [b]	3.43 [c]	3.46 [c]	3.38 [b]
Total solids (°Brix)	10.10 [a]	13.40 [b]	14.10 [c]	15.30 [d]	13.10 [b]
Color, *L**	**87.35** [a]	74.00 [b]	64.99 [c]	59.44 [d]	75.06 [b]
*a**	-1.31 [a]	0.71 [b]	0.30 [d]	0.29 [d]	-1.82 [b]
*b**	8.33 [a]	19.20 [b]	21.17 [c]	21.32 [c]	18.76 [b]
Overrun (%)	**54.62** [a]	**48.72** [b]	**36.48** [c]	**25.59** [d]	**53.81** [a]
Hardness (N)	**0.88** [a]	**1.26** [b]	**1.24** [b]	**1.29** [b]	**1.16** [c]
Viscosity (cp)	**53.00** [a]	**57.50** [a]	**67.6** [b]	**75.35** [c]	**63.70** [b]

Analyses	Freeze-dried Yanang powder				Yanang juice 10%
	0%	10%	20%	30%	
Melting rate (mL/min)	**1.50** [a]	**1.32** [b]	**1.19** [c]	**1.09** [c]	**1.42** [a]

Results are presented as mean ± SD (n = 3).
Data superscripted with a-d in the same row are significantly different (p< 0.05).

Lower melting rate relates to the sustainability of the sherbet's shape, which is typically evaluated as a good quality. The melting rate of sherbet with higher concentration was +deceased which showed the effect of freeze-dried powder on the melting rate property of sherbet. Hardness and viscosity were also affected by this effect. The values had higher when added more freeze-dried powders compared to the control and sherbet with fresh extract.

Table 4. Antioxidant activity of sherbet samples containing 0, 10%, 20% 30% of Yanang powder and 10% fresh Yanang extract.

Analyses	Freeze-dried Yanang powder				Yanang juice 10%
	0%	10%	20%	30%	
TPC (mg GAE/g)	0.52 [a]	1.64 [c]	1.97 [d]	2.02 [d]	0.69 [b]
DPPH (mg Trolox equivalent/g)	2.33 [a]	4.01 [b]	4.93 [c]	5.66 [d]	2.52 [a]
FRAP (mg GAE/g)	0.034 [a]	0.041 [a]	0.054 [b]	0.067 [c]	0.039 [a]

Results are presented as mean ± SD (n = 3).
Data superscripted with a-d in the same row are significantly different (p< 0.05).

In this study, Yanang sherbet was examined to determine total phenolic content. Among samples, 30% Yanang powder added sherbet yielded highest TPC (2.02 mg GAE/g). The TPC values of the sherbet were lower, being 1.97 and 1.64 mg GAE/g for 20% and 10% Yanang powder added in sherbets, respectively (Table 4). However, fresh Yanang extract added into sherbet has lower TPC compared to sherbet with 10% Yanang powder. It concluded that the encapsulation of bioactive compounds via maltodextrin preserve antioxidants during thermal processing.

Results of antioxidant activity determined by DPPH and FRAP assays were shown in Table 4. Free radical scavenging property of Yanang sherbet samples was characterized by a higher free radical scavenging property (5.66 mg/g), with comparison to those of sherbet with fresh extract and control (2.52 and 2.33 mg/g), respectively.

Reducing power obtained by FRAP assay serves as a significant indicator of its antioxidant activity of sherbet. The reducing power of freeze-dried Yanang powder sherbet was in range from 0.041 to 0.067 mg GAE/g, which were higher than fresh extract sherbet and control. The highest amount of Yanang added into sherbet had the highest TPC value corresponded with higher DPPH and FRAP values.

Results of sensory evaluation were shown in Table 5. The sensory properties of sherbet samples in terms of appearance/color, texture, flavor, foreign taste and overall acceptability were significantly affected by the addition of Yanang powder. According to the panelists, undesirable sensory taste perception characteristic (as known as foreign taste) of Yanang was reported and this flavor was increased with increasing Yanang powder concentrations with negative effect on the sensory scores. The results suggested that the addition of freeze-dried Yanang powder at 10% had the highest acceptability among sherbet products (20% and 30%) compared with fresh Yanang extract sherbet as indicated by the higher scores.

Table 5. The scores of the sensory properties of the sherbet samples containing 0, 10%, 20% 30% of Yanang powder and 10% fresh Yanang extract.

Sensory Properties	Freeze-dried Yanang powder			Yanang juice 10%
	10%	20%	30%	
Appearance/color	**7.83^{a}**	**5.70^{b}**	**5.57^{b}**	**6.70^{c}**
Texture	**6.97^{a}**	**5.77^{b}**	**5.87^{b}**	**5.97^{b}**
Flavor	**6.90^{a}**	**5.40^{b}**	**5.40^{b}**	**5.83^{b}**
Foreign taste	**6.07^{a}**	**5.47^{b}**	**5.33^{b}**	**5.67^{ab}**
Overall acceptability	**7.73^{a}**	**5.93^{bc}**	**5.67^{c}**	**6.50^{b}**

Results are presented as mean ± SD (n = 3).
Data superscripted with a-d letter within the same row are significantly different *$p<0.05$*).

4. Microbial analysis

The microbial counts in sherbet samples may have resulted from inadequate processing especially when adding fresh plant solution, which may lead to multiplication of microorganisms present in ice cream immediately after pasteurization [21].

Table 6. Total viable count (TVC) of the sherbet samples containing 0, 10%, 20% 30% of Yanang powder and 10% fresh Yanang extract.

Sensory Properties	Freeze-dried Yanang powder			Yanang juice 10%
	10%	20%	30%	
TVC (cfu/g)	$< 2.5x10^{2}$	$< 2x10^{2}$	$< 2x10^{2}$	$< 1.2x10^{4}$

Cfu means colony-forming unit per 1g of sherbet.

After 1 month of storage, it was observed that the TVC values for all the sherbet samples were less than $1.2x10^{4}$ cfu/g (Table 6). According to Thailand Notification of the Ministry of Public Health (2001) vol.222, TVC of ice cream and sherbet products should not exceed 600,000 cfu/g.

CONCLUSION

The use of freeze-drying technique to produce Yanang powder resulted in very high yield, which there is no product loss during freeze drying. Maltodextrin was added to increase the glass transition temperature of the powder, thus the end result showed that the yield of freeze drying is satisfied. The water activity of freeze dried Yanang powder was lower than 0.50 therefore the powder would have lower risk of spoilage due to microbial reactions after fortified into food products. As the results of total phenolic content, Trolox equivalent and gallic equivalent of freeze-dried Yanang powder, it indicated that the destruction of total phenolic content and antioxidant compounds was protected under freeze drying process. After adding Yanang samples into sherbet, the increased Yanang powder concentration increased pH values and total soluble solid of the sherbet. The color parameters of Yanang powder sherbet trended to decrease and the most concentration showed the lowest $L*$ values. The same results found for $a*$ and $b*$. The melting rate of sherbet with higher concentration deceased indicating the effect of freeze-dried powder on property of sherbet. Hardness and viscosity were also affected by this factor, which showed the higher values when added more freeze-dried powder compared to the control and sherbet with fresh extract. The highest amount of Yanang added into sherbet had the highest TPC value corresponding with higher DPPH and FRAP values. The sensory was significantly affected by the addition of Yanang.

At 10% of freeze-dried Yanang powder adding had the highest acceptability scores compared with others. Moreover, TVC values for all sherbet samples after 1 month were less than the limitation. These results based on sensory evaluation, showed the appropriate amount of freeze-dried Yanang powder fortified in sherbet was 10% compared with fresh Yanang extract at the same level.

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