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Research Paper / Araştırma Makalesi

Effect of Using Green Tea (*Camellia sinensis* L.) Powder and Probiotic Bacteria on Probiotic Shelf Life and Quality Properties of Ice Cream

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ABSTRACT

In this study, the effect of green tea powder (GTP) in ice cream production on the ice cream properties and viability of probiotic bacteria [*Bifidobacterium animalis* ssp. *lactis* (*Bifidobacterium* BB-12) (P)] was investigated. Four ice cream groups (Control, C: without GTP and P; Probiotic Control (PC): with P; P+1% GTP; P+3% GTP samples) were produced. *Bifidobacterium* BB-12 (P) was added at 10⁸ cfu/mL. pH, overrun, color, first dripping time, complete melting time and sensory properties were carried out on the second day. P was counted at 2, 15, 30, 45, 60, 75 and 90 days of storage. *Bifidobacterium* BB-12 number of PC was 6.54 log cfu/g at the 90th day. *Bifidobacterium* BB-12 number of PC was 7.10 log cfu/g, P+1% GTP sample was 6.48 log cfu/g and P+3% GTP sample was 5.84 log cfu/g at the 75th day. *Bifidobacterium* BB-12 number of PC was 7.24 log cfu/g, P+1% GTP sample was 6.59 log cfu/g and P+3% GTP sample was 6.69 at the 60th day. Sensory properties and probiotic counts showed that P+1% GTP sample could be accepted as a probiotic ice cream until day 75. Hence, GTP could be successfully used as a functional ingredient in the production of probiotic ice cream.

Keywords: Ice cream, *Bifidobacterium* BB–12, Green tea powder, Shelf life, Functional food

Yeşil Çay (*Camellia sinensis* L.) Pudrası ve Probiyotik Bakteri Kullanımının Dondurmanın Probiyotik Raf Ömrü ve Kalite Özelliklerine Etkisi

ÖΖ

Bu çalışmada, dondurma üretiminde yeşil çay pudrası (YÇP) kullanımının probiyotik bakterinin (*Bifidobacterium* animalis ssp. lactis, Bifidobacterium BB-12) (P) canlılığı ve dondurma özelliklerine etkileri araştırılmıştır. Bu amaçla dört çeşit dondurma örneği [(Kontrol: YÇP ve probiyotik bakteri içermeyen örnek; Probiyotik Kontrol (PK): probiyotik bakteri içeren örnek; probiyotik bakteri ve %1 YÇP içeren örnek (P+%1 YÇP); probiyotik bakteri ve %3 YÇP içeren örnek (P+%3 YÇP) üretilmiştir. Probiyotik grup örneklerin üretiminde 10⁸ kob/mL *Bifidobacterium* BB-12 ilave edilmiştir. Örneklerde pH, hacim artışı, renk, ilk damlama süresi ve tam erime süresi ile duyusal özellikler üretimin 2. gününde belirlenmiştir. Probiyotik bakteri sayısı depolamanın 2, 15, 30, 45, 60, 75 ve 90. günlerinde tespit edilmiştir. PK örneğinde 90. günde *Bifidobacterium* BB-12 sayısı 6.54 log kob/g olarak bulunmuştur. Probiyotik bakteri sayıları 75. günde PK kodlu örnekte 7.10 log kob/g, P+%1 YÇP kodlu örnekte 6.48 log kob/g ve P+%3 YÇP kodlu örnekte 5.84 log kob/g olarak saptanmıştır. Depolamanın 60. gününde ise PK kodlu örnekte 7.24 log kob/g, P+%1 YÇP kodlu örnekte 6.59 log kob/g ve P+%3 YÇP kodlu örnekte 6.69 log kob/g olarak bulunmuştur. Sonuç olarak; duyusal özellikler ve probiyotik bakteri sayıları dikkate alındığında P+%1 YÇP kodlu örnekte 5.9 log kob/g ve P+%3 YÇP kodlu örnekte 6.69 log kob/g olarak bulunmuştur. Sonuç olarak; duyusal özellikler ve probiyotik bakteri sayıları dikkate alındığında P+%1 YÇP kodlu örnekte 5.9 log kadar probiyotik raf ömrünü koruduğu ve yeşil çaylı probiyotik dondurma olarak kabul edilebileceğini göstermiştir. Bu nedenlerle, YÇP'nın probiyotik dondurma üretiminde fonksiyonel bir bileşen olarak başarıyla kullanılabileceği söylenebilir.

Anahtar Kelimeler: Dondurma, Bifidobacterium BB-12, Yeşil çay pudrası, Raf ömrü, Fonksiyonel gıda

INTRODUCTION

Consumer perceptions to foods are important in the development and marketing of products [1]. Because the relationship between nutrition and health has a huge impact on consumer's nutrition preference, this has led to the development of functional food concepts, which have positive health effects as well as nutritional value [2]. For this reason, consumers use foods containing natural antioxidants, dietary fibers, natural colorants, low calories, low fat and other natural additives. In addition, prebiotic or probiotic bacteria supplementation to ice cream provides functional properties and can increase the nutritional value and health benefits of ice cream [3]. Dairy products are in the appropriate composition for the survival of probiotics [4-6]. The viability of probiotic culture in the food product should be adequately high, 10⁶-10⁸ cfu/mL or g during the shelf life [6-8]. During the production of ice cream, the viability of probiotic strains is significantly preserved. In particular, the effect of lowstorage probiotic temperature and bacteria supplementation on flavor, texture and other sensory properties is very low [9]. Ice cream mix basically include fat, protein, sugar, stabilizers, and emulsifiers [10]. It can be supplemented with probiotics and other active components providing additional functional properties. So, nutritional enrichment of ice cream is an effective way to increase the perception of health [1] and adding green tea is thought to be a good way for producing a new kind of ice cream. Tea is used in green, oolong and black tea forms, obtained from the leaves of the Camellia sinensis L plant. Green tea is healthier because it is the least processed form. Green tea is very beneficial because of the phenolic compounds, especially catechins, which is powerful antioxidant effects and health benefits [11-14]. The nutritional significance of green tea is attributed to the phenolic compounds (30% dry weight). Green tea contains proteins (15-20% dry weight, dw), fiber (26% dw), carbohydrates (5-7% dw, such as cellulose, pectin, glucose, fructose, sucrose), lipids (7% dw), linoleic and α-linolenic acids, minerals (5% dw, such as Ca, Mg, Mn, Fe, Zn, etc.) and vitamins (such as B, C, E), chlorophyll and carotenoids, volatile compounds and etc. [11].

This study aim was to investigate the effect of green tea powder (GTP) supplementation on the viability of *Bifidobacterium* BB-12 added during the freezing process of ice cream. It was also to determine the physical and sensory properties of ice cream samples. In this way, the development of a new type of ice cream with natural antioxidant, natural green color and probiotic bacteria will be more nutritious and functional in addition to developing different ice cream formulations.

MATERIALS and METHODS

Materials

Whole cows' milk and cream were supplied from the Dairy Plant of Atatürk University (Erzurum, Turkey). Green tea (*C. sinensis*) powder (GTP) was purchased from Atatürk Tea and Horticultural Research Institute (Rize, Turkey). The particle size of GTP used as

supplement was less than 355µm. The average dry matter (%), ash (%), protein (%) and pH of GTP were found, respectively, as follows: 95.47, 4.13, 21.50 and, 5.20. The GTP found in glass bottle was pasteurized in a water bath at 65°C for 30min and stored in the refrigerator (4°C) until use. After pasteurization, no coliform bacteria were detected in the GTP sample (<1 log cfu/g). The freeze-dried lactic culture for Direct Vat Set (DVS) Bifidobacterium animalis ssp. lactis BB-12, Bifidobacterium BB-12 (NuTrish® BB-12®) (P) was from Chr-Hansen (İstanbul, obtained Turkey). Commercial sugar, stabilizer (salep) and emulsifier (mono glyceride) were supplied from a local market (Erzurum, Turkey) and skimmed milk powder by Pinar Dairy Products Co. (Izmir, Turkey).

Ice Cream Mix Preparation and Production of Ice Cream Samples

Whole cow milk was divided into four equal parts of 4 kg each. The fat content of ice cream mix samples was adjusted to 5%. The dry matter ratio of milk was increased by the addition of nonfat milk powder. The content of sugar (15%), fat (5%), non-fat milk solids stabilizer 0.7%) and (11%), (salep, emulsifier (monoglyceride, 0.2%) were the same in all ice cream mixes. For all the parts mix preparation procedure and pasteurization were same. One part was control sample (C) (without GTP and P), the second one was probiotic control (PC) (containing only P), the third one was containing P and 1% GTP (P+GTP1 sample) and the fourth part was containing P and 3% GTP (P+GTP3 sample) (Figure 1). Mixes were frozen in the ice cream machinery (-5°C) (Ugur Co., Nazilli, Turkey). The cylinder capacity of the freezer was 6 L and 4 L mix was added to allow for overrun development. The whipping time was 15 min to get to -5°C.

Analyses

Physical, chemical, color and sensory analyses were done after two-day storage but probiotic bacteria evaluation was conducted for 90 days. The experimental procedure was duplicated.

Physical and Chemical Analyses

For overrun (OR), A 100-mL cup was used and values were calculated using the following formula [15]:

Overrun (%) = (weight of the ice cream mix – weight of same volume of the ice cream sample) / (weight of same volume of the ice cream sample) \times 100

First dripping time and complete melting time were determined according to Guven and Karaca [16]. pH values of the samples were determined according to AOAC [17]. The chemical parameters evaluated were protein (Kjeldahl method), and Fat (Gerber method) of ice cream samples were determined according to Gürsel and Karacabey [18].

Color Measurement

The color measurements of the ice cream samples were determined with a Minolta colorimeter CR-200 (Minolta

Camera Co., Osaka, Japan) according to the method suggested by Cakmakci et al. [19].



Figure 1. Processing steps for ice cream production *Probiotic: *Bifidobacterium animalis* ssp. *lactis* (*Bifidobacterium* BB-12) **GTP: Green tea powder

Bifidobacterium BB-12 Count

The number of viable probiotic bacteria was determined at 2, 15, 30, 45, 60, 75 and 90 days of frozen storage at $-20\pm1^{\circ}$ C. 10 g ice cream sample were serially diluted (w/v) up to 10^{-7} with sterile 0.85% NaCl solution. Then, spread-inoculated in duplicate onto plates of de Man, Rogosa, Sharpe agar (MRS agar, Merck) for *Bifidobacterium* BB-12. The inoculated plates were incubated in anaerobic jars for 72h at 35±1°C anaerobically using Anaerocult A (Merck, Darmstadt, Germany).

Sensory Analysis

For the sensory assessment of the ice cream samples, fifty consumer panelists were chosen. The panel of assessors was an external panel of non-smokers who were very familiar with ice cream products [20]. Panelists were students or academic staff of Ataturk University Food Engineering Department (Erzurum, Turkey). The samples were assessed for color, gumminess, texture, flavor, sweetness, and general acceptability. Point scale ranging from poor (1) to excellent (9) was used for sensory evaluation. For color, texture, flavor, and general acceptability criteria; A score of 9-8 means very good, 7-6 is good, 5-3 is average and 2-1 means bad. For the chewiness and sugariness criteria, 9-8 means normal/ideal, 7-6 means less, 5-3 means more, and 2-1 means no/not enough. During the sensory analysis, panelists drank warm water to neutralize the mouthfeel between samples.

Statistical Analysis

Completely randomized factorial design was used to conduct the experiments, which included the four ice cream sample types (C, PC, P+GTP1 and P+GTP3), seven storage periods (2, 15, 30, 45, 60, 75, 90 days) and two replicates. Statistical analyzes were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA) software.

RESULTS and DISCUSSION

Physical and Chemical Properties

The pH, protein and fat content results of ice cream samples are shown in Table 1. The pH values of PC and P+GTP1 samples were not different statistically but the other samples were different (p<0.05). GTP and probiotic bacteria addition decreased pH when compared to the Control sample. Probiotic bacteria are lactic acid bacteria and they lower the pH of the ice cream as they are added to the mix. The pH of the GTP

used in the research is also 5.2 and it is lower than the pH of the mix. For these reasons, the pH of the samples can be listed as follows: C > PC > P+GTP1 > P+GTP3. GTP addition caused increases in the protein content as the relative of ice creams compared to the Control and Probiotic Control samples. As the amount of GTP added increased, the protein content of the ice cream also increased. This result can be explained by the high ratio of dry matter (95.47%) and protein (21.5%) of GTP. As the amount of GTP added increased, the literature that green tea contains 7% lipid [11], it is thought that this amount may cause a slight decrease in the fat content of ice creams.

Table 1.	The ph	ysical pro	perties of	ice cream	samples	produced
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lce cream	Properties ²					
samples ¹	pН	Protein (%)	Fat (%)			
С	6.48±0.03 ^a	5.14±0.19 ^b	5.15±0.0.07 ^{ab}			
PC	6.27±0.01 ^b	4.79±0.04°	5.20±0.14ª			
P+GTP1	6.25±0.01 ^b	5.36±0.14 ^{ab}	5.05±0.07 ^{ab}			
P+GTP3	6.19±0.01 ^c	5.68±0.04 ^a	4.93±0.04 ^b			

¹C, Control sample (no GTP and P); PC, Probiotic control (containing only P); P+GTP1: containing P and 1% GTP; P+GTP3: containing P and 3% GTP; ²Means \pm standard deviation followed by different letters in the same column are significantly different (*p*<0.05).

Overrun is a defined an increase in volume of ice cream during processing and is related to yield and profit to the producer [21]. Overrun values were significantly different between samples (p<0.05) and varied from 18 to 40 % (Table 2). The lowest overrun was obtained in P+GTP1 whereas the highest was in the PC sample. The increased level of GTP decreased the overrun value. This result was similar to studies by Goraya and Bajwa [21] who added 'Indian gooseberry', Çakmakçı et al. [19] who added oleaster flour and crust, and Yangılar [22] who added banana flour and pulp in ice cream. Gorava and Bajwa [21] explained that the increasing level of gooseberry decreased overrun by weakening air incorporation. Akalın et al. [23] reported that low overrun values (varied 20.7-39.2%) could be because of using a batch type freezer for freezing process and composition of ice creams. In another study, the increased cassava

bagasse content decreased the overrun and could be the reason for the increase in viscosity of the mix, decreasing the molecular mobility, which made incorporation and uniform distribution of the air cells difficult [24]. Bahramparvar et al. [25] found that the overrun value of ice creams produced with some gums was changed between 18.8% to 28.6% and they thought that the low overrun could be related to the inefficiency of the ice cream maker in air incorporation and excessive time required for freezing. On the other hand, air in ice cream gives a light texture and effects the melt down and hardness; not only amount of air incorporated but also the distribution of size of the air cells influences these parameters [26]. The properties of ice cream depend mostly upon the components used as well as manufacturing parameters and storage [26-28].

Table 2.	The tech	nological	and c	color p	proper	ties of	ice	cream	samp	les

lce cream samples ¹	Technological Properties ²			Color parameters			
	Overrun (%)	First dropping time (s)	Complete melting time (s)	L*	a*	b*	
С	37.9±0.11 ^b	3620±84.8 ^a	5430±42.4 ^a	85.62±0.90 ^a	3.16±0.09 ^a	9.42±0.01°	
PC	40.5±0.10 ^a	2040±28.3°	5005±21.2 ^b	84.19±0.95ª	3.07±0.06 ^a	9.61±0.28 ^c	
P+GTP1	29.0±1.00 ^c	2610±42.4 ^b	3900±84.8 ^d	65.00±0.99 ^b	6.20±0.03 ^c	25.10±0.44 ^b	
P+GTP3	18.4±0.54 ^d	2760±84.8 ^b	4080±84.8 ^c	54.06±0.35°	5.08±0.72 ^b	26.14±0.10 ^a	

¹C, Control sample (no GTP and P); PC, Probiotic control (containing only P); P+GTP1: containing P and 1% GTP; P+GTP3: containing P and 3% GTP; ²Means \pm standard deviation followed by different letters in the same column are significantly different (*p*<0.05).

The first dropping and complete melting time values are given in Table 2. The highest first dropping time belonged to the control sample and the lowest one was the probiotic control sample. GTP caused longer first dropping time to the PC sample and but the samples with GTP melted more rapidly than the PC and P samples, respectively. As the GTP ratio increased, the first dropping time and complete melting time became

longer in P+GTP1 and P+GTP3 samples (Table 2). The adding of banana peel flour affected the first dropping time positively and increasing of banana pulp caused longer first dropping times [21]. P+GTP3 sample had more protein content than P+GTP1 due to high protein content (21.5%) of GTP used in this study and this protein content can be reason for difference in melting properties. It was reported that protein structure may influence the viscosity of the ice cream mixes although total solid, fat, and protein concentrations were held constant in ice cream mix [29,30]. In addition to high protein content of green tea, drying of the leaves at high temperatures (above 100°C) can be another reason. Because of being partially denatured proteins often show important hydrophobic interactions, and the structure of the continuous phase is strengthened, causing increased amounts of bound water. Two different stevia varieties (Stevia rebaudiana Morita and Criolla) and extract concentrations (5% and 8%), which were used in ice cream production, had significant influence on overrun and it was shown that the higher the concentration of extract, the lower the percentage of yield of whipping in both varieties. In the same study, the ice cream made with S. rebaudiana Criolla sowed less tendency to melt. Authors stated that the low or rapid melting was generally linked to the amount of sweetener, stabilizer and/or emulsifier [30]. Muse and Hartel [32] showed that the extent of fat destabilization and ice crystal size had the largest effect, but overrun was not an effective factor in the melting rate and it was caused by the thickener and plasticizer effects of high levels of sugar in solution. Although slower melting rate in the ice creams with higher overruns was attributed to a lower heat transfer rate due to the larger air volume, it was also stated that it could be due to the more circuitous flow of the melting liquid [32]. Sakurai et al. [33] also reported that ice cream with high overrun began to melt slowly and showed good resistance to melting.

Color Properties

Consumers prefer natural colors, especially processed foods. Table 2 shows the color values (L^* , a^* , b^*) of the ice cream samples. The L^* , a^* , and b^* color values were influenced by the addition of GTP. These values significantly differed between samples (p <0.01). As

expected, the addition of GTP increased the redness and yellowness of ice creams but decreased the lightness. Similarly; Hezer [34] determined that the addition of purslane (*Portulaca oleracea* L.) (5, 10 and 15%) decreased L^* and a^* values and increased b^* values in ice cream samples. According to Silva Faresin et al. [35], the addition of *Spirulina platensis* powder (1%) decreased the L^* (53.95-57.17), a^* (-9.02 and -7.40), and b^* (17.99-22.11) values of ice cream samples. On the other hand, there was no statistically significant difference in control samples (C and PC). The most similar L^* value to the control samples was found in P+GTP1 sample. In sensory analysis, P+GTP1 sample with a light green color was preferred than P+GTP3 sample (Table 2).

Viability of Bifidobacterium BB-12

The viable Bifidobacterium BB-12 counts are shown in Table 3. Bifidobacterium BB-12 count showed significant differences (p < 0.05) among the samples and ranged from 7.50 to <2 log cfu/g among the samples. The highest average Bifidobacterium BB-12 count was found in the PC group. PC samples retained probiotic characteristics during 90 days (>10⁶ cfu/g). Probiotic bacteria generally show good survivability in ice cream up to the end of shelf life [5,9]. Generally, the addition of GTP reduced the number of Bifidobacterium BB-12 according to the PC sample, but probiotic properties were retained up to 75 days with P+GTP1 and 60 days with P+GTP3. Perhaps, probiotic viability may have decreased during the ice cream processing, due to the lengthening of the process for more homogeneous mixing of the green tea powder. The researches have shown that the counts of probiotic bacteria decrease significantly throughout storage. However, it was stated that mixing and freezing processes during the conversion of the mixture into ice cream had a greater effect on the reduction of probiotic bacteria viability [9]. When the results are evaluated collectively; It can be said that the ice cream sample (P+GTP1) produced with the addition of 1% GTP and Bifidobacterium BB-12 preserves the probiotic ice cream criteria for 75 days (Table 3). As probiotic ice cream samples, PC>90 days, P+GTP1 =75 days and P+GTP3 = 60 days were found to have probiotic properties (Table 3).

Table 3. *Bifidobacterium* BB-12 counts of ice cream samples (log cfu/g)

Storage (day)	Ice cream samples ¹					
Storage (day) -	PC ²	P+GTP1	P+GTP3			
2	7.50±0.50 ^a	7.00±0.03 ^{a-e}	7.00±0.00 ^{a-e}			
15	7.30±0.30 ^{ab}	7.00±0.00 ^{a-e}	6.85±0.15 ^{b-e}			
30	7.49±0.09 ^a	6.95±0.05 ^{a-e}	6.89±0.11 ^{b-e}			
45	7.39±0.05 ^{ab}	6.93±0.08 ^{b-e}	6.93±0.07 ^{b-e}			
60	7.24±0.24 ^{a-c}	6.59±0.11 ^{de}	6.69±0.09 ^{c-e}			
75	7.10±0.02 ^{a-d}	6.48±0.00 ^e	5.84±0.09 ^f			
90	6.54±0.27 ^{d-e}	5.86±0.09 ^f	-			

¹Means ± standard deviation followed by different letters in the same column are significantly different (p<0.05); ²PC, probiotic control (containing only P); P+GTP1: containing P and 1% GTP; P+GTP3: containing P and 3% GTP

Sensory Properties

The sensory characteristics of ice cream samples are shown in Table 4. The ice creams with probiotic bacteria has been found to have little effect on its flavor, texture or other sensory characteristics. A similar result was also reported by Mohammadi et al. [9]. But, the addition of the GTP significantly affected the sensory qualities of the samples. Color scores were significantly different, between 6.52 and 8.32. The highest score was determined in Control. Other samples are different statistically. GTP gave a greenish color to ice cream that was desirable for some panelists, especially 1% GTP addition. The samples containing GTP showed relatively low scores in terms of organoleptic qualities compared to the C and PC groups. However, in general, PC and P+GTP1 samples were found to be statistically similar (Table 3). The C sample is considered to have higher scores due to the usual color, texture, sweetness, etc. of the ice cream. Panelists reported that P+GTP3 sample had a mild texture and melted easily. Also, 3% GTP negatively affected all the sensorial properties. Panelists indicated that as GTP amount increases in the ice cream samples, they felt bitterness; especially the 3% GTP was more bitter. This bitterness is due to the composition of green tea and it is felt more as the usage rate increases. P+GTP1 sample was favored by the panelists after C and PC samples.

Table 4. Sensorial properties of ice cream samples

Ice cream samples ¹	Color ²	Texture	Gumming structure	Flavor	Sweetness	General acceptability		
С	8.3±0.0 ^a	7.8±0.0 ^a	7.5±0.2 ^a	7.6±0.1 ^a	8.0±0.0 ^a	7.8±0.3 ^a		
PC	8.2±0.2 ^{ab}	7.6±0.2 ^a	7.1±0.1⁵	7.0±0.5 ^{ab}	7.6±0.2 ^{ab}	7.5±0.2 ^a		
P+GTP1	7.6±0.2 ^b	7.7±0.0 ^a	7.1±0.2 [♭]	6.1±0.5 ^b	7.4±0.2 ^b	6.8±0.2 ^a		
P+GTP3	6.5±0.3 ^c	7.3±0.1 ^b	6.6±0.1 ^c	4.4±0.3 ^c	6.1±0.1 ^c	4.8±0.8 ^b		

¹C, control sample (no GTP and P); PC, probiotic control (containing only P); P+GTP1: containing P and 1% GTP; P+GTP3: containing P and 3% GTP; ²Means \pm standard deviation followed by different letters in the same column are significantly different (*p*<0.05).

CONCLUSIONS

In the production of probiotic ice cream with Bifidobacterium BB-12, and 1% GTP supplement, the probiotic property of the ice cream was preserved for up to 75 days. The ice cream containing 1% GTP were preferred by the panelists in respect to sensorial properties following the C and PC samples. In general, PC and P+GTP1 samples were found to be statistically similar. Given GTP's nutritive content and functional properties, it may be used as a suitable of natural additive in ice cream production to enhance nutritional value, antioxidant capacity and natural greenish color. Overall evaluation of the results led to the conclusion that 1% GTP may be a suitable natural additive in probiotic ice cream formulation in spite of its slight bitterness. However, conscious consumers, who know the benefits of green tea, probiotics, and health effect said that a slight bitterness caused by tea in ice cream with 1% GTP is not important.

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