

NUTRIENT COMPOSITION, ANTIOXIDANT POTENTIAL AND SENSORY EVALUATION OF DEVELOPED MIXED CONCENTRATED JUICE

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Graphical Abstract



Abstract

The purpose of this study is to determine nutrient and antioxidant content, and consumer acceptance of mixed concentrated juice of three ingredients namely pomegranate, honey and date extracts. The nutrient content was analysed based on AOAC method. The antioxidant was determined using Folin-Ciocalteu, DPPH and FRAP assays. Sensory analysis was carried out on 30 untrained panels using hedonic scale. Each 100 g juice provided 380 kcal, contained 55.65 g sugar, 92.13% carbohydrate, 1.97% protein, 0.39% fat, 270.70 mg potassium, 58.43 mg sodium, and 31.58 mg magnesium. The juice contained total polyphenol of 1752.10 mg GAE/100 ml, and antioxidant content of 12.80 mM TE/ml (DPPH) and 51.681 mM TE/ml (FRAP). As for the sensory evaluation, there was no significant difference ($P>0.05$) in the mean score for the attributes of this newly developed mixed concentrated juice compared to the commercial ones. It can be concluded that the juice has a high nutrient and antioxidant content. The taste of the juice was acceptable among consumers. The combination of more than one type of food could increase the nutrient value of the foods but further studies are needed to see the synergistic effect provided by each food.

Keywords: Product development, antioxidant, pomegranate, date, honey

Abstrak

Tujuan kajian ini adalah untuk menentukan kandungan nutrisi, antioksidan dan penerimaan jus campuran pekat berasaskan tiga bahan iaitu ekstrak delima, madu dan kurma. Kandungan nutrisi dianalisa menggunakan kaedah AOAC. Kandungan antioksidan ditentukan menggunakan kaedah Folic-Ciocalteu, DPPH dan FRAP. Analisis sensori dijalankan ke atas 30 orang panel tidak terlatih menggunakan skala hedonik. Setiap 100 g jus ini mengandungi 380 kcal, 55.65 g gula, 92.13% karbohidrat, 1.97% protein, 0.39% lemak, 270.70 mg kalium, 58.43 mg natrium dan 31.58 mg magnesium. Jus ini mengandungi jumlah kandungan polifenol (1752.10 mg GAE/100ml) dan antioksidan 12.80 mM TE/ml (DPPH) dan 51.681 mM TE/ml (FRAP). Penilaian sensori ke atas jus menunjukkan tiada perbezaan signifikan ($P>0.05$) bagi purata skor atribut untuk jus campuran pekat yang baru dibangunkan berbanding jus campuran komersial. Kesimpulannya jus ini tinggi kandungan nutrien dan antioksidan. Rasa jus ini diterima baik oleh pengguna. Campuran lebih dari satu jenis makanan dapat meningkatkan kandungan nutrien sesuatu makanan namun kajian selanjutnya perlu dijalankan bagi melihat kesan sinergistik yang dihasilkan oleh setiap makanan.

Kata kunci: Pembangunan produk, antioksidan, delima, madu, kurma

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1.0 INTRODUCTION

Dietary supplementation from functional foods especially from fruits, vegetables, and herbs has gained attention among consumers. Fruits and vegetables are a rich source of micronutrients and phytochemicals, and their consumption is linked with non-communicable disease risk reduction [1]. The intake of fruits and vegetables among the global population is lower than the recommended intake which is a minimum five servings of fruits and/or vegetables daily [2, 3]. Although most people are aware of health, a hectic life leaves most of them with less time to prepare and consume a healthy diet. As a substitute, they have chosen dietary supplements as an alternative for them in order to be healthy. Fruits and vegetables are also good sources of antioxidants. Natural antioxidants present in foods are linked with lower risk of various chronic diseases such as cardiovascular disease and obesity [4]. Antioxidants act by protecting body cells against free radical damages [5]. Free radicals are unstable molecules that contain an unpaired electron that makes them highly reactive, and they will steal electrons from other molecules in a process called oxidation which causes damage to the structure of the molecules, and contributes to various problems over time such as aging and degenerative disease [5, 6]. Free radicals are byproducts of normal body metabolism, and can also be from the environment such as pollution, cigarette smoke, medication, and radiation [5].

Food such as pomegranate, date and honey are nutritious, and have various health benefits [7]. Pomegranate, date, and honey have previously been reported to have high polyphenol and antioxidant content [8, 9, 10]. Polyphenols, a phytochemical found in abundance in plant-based foods, provide antioxidant properties, and protection against various types of diseases such as neurodegenerative disease, cancer, diabetes, osteoporosis, and cardiovascular disease [11]. There are more than 8000 identified polyphenols found in foods [12]. Pomegranate juice contains three times higher antioxidant activity compared to green tea and red wine [13]. Besides having a high antioxidant content, pomegranate, date, and honey are also good sources of energy, and other macro and micronutrients [9, 14]. The aim of this study is to determine the nutrient, antioxidant, and polyphenol content of a newly developed mixed concentrated juice, and also to determine the sensory acceptance among consumers.

2.0 METHODOLOGY

2.1 Selection of Ingredients

Three main ingredients used in the newly developed mixed concentrated juice were honey, date, and

pomegranate. The honey was collected from East-Coast Peninsular Malaysia. Meanwhile, the date and pomegranate were bought from a local market located in Kuala Lumpur.

2.2 Chemicals and Reagents

Ethanol, acetone, acetate buffer, amyloglucosidase, petroleum ether, boric acid, sodium hydroxide, potassium sulphate, copper sulphate, and mineral stock solution (calcium, ferum, potassium, magnesium, and zinc) were purchased from System ChemAR. Folin-Ciocalteu phenol reagent, sodium carbonate, Gallic acid, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Trolox, methanol, 2,4,5-Tripyridyl-s-triazine (TPTZ), ferric chloride, hydrochloric acid, sodium acetate, and acetic acid were obtained from Sigma-Aldrich, USA.

2.3 Proximate Analysis

The moisture, ash, protein, fat, and carbohydrate content were analysed using standard AOAC [15] method.

2.3.1 Moisture Content

The moisture content was determined by using the oven-drying method. Samples (2 g) were weighed using an analytical balance (Mettler Toledo, Switzerland), and dried overnight in the oven at 105 °C to a constant weight, and placed in a desiccator to completely cool prior to weighing. The moisture content was determined by using the following formula:

$$\text{Moisture content (\%)} = \frac{\text{Weight of wet sample (g)} - \text{Weight of dried sample (g)}}{\text{Weight of wet sample (g)}} \times 100\% \quad (1)$$

2.3.2 Ash Content

Total ash content was determined using dry ash method. The crucibles containing samples (3 g) were placed on a hotplate and heated until the samples turned black and stopped producing white fumes. The samples were then placed in a muffle furnace overnight at 550 °C, and cooled in a desiccator prior to weighing. The ash content was determined using the formula:

$$\text{Total ash content (\%)} = \frac{\text{Weight of crucible with ash (g)} - \text{Weight of crucible (g)}}{\text{Weight of dried sample (g)}} \times 100\% \quad (2)$$

2.3.3 Crude Fat

The crude fat content was determined using the Soxhlet method. Samples (2 g) were placed in a thimble, and 70 ml petroleum ether was added to the extraction cup. The process of fat extraction from samples was carried out using Soxtec System HT6. The

extraction cups were then dried in the oven for 15 minutes, and transferred to desiccators to cool prior to weighing. The crude fat content was determined using the formula:

$$\text{Crude fat content (\%)} = \frac{(\text{Weight of extraction cup + fat (g)} - \text{Weight of extraction cup (g)})}{\text{Weight of wet sample (g)}} \times 100\% \quad (3)$$

2.3.4 Crude Protein

The crude protein content was determined using the Kjeldahl method. About 7 g potassium sulphate, and 2 g copper sulphate were added to a digestive tube containing sample (2 g). About 12 ml concentrated sulphuric acid was added to the tube, and heated in Tecator Digestion System for one hour until the solution turned a greenish blue colour. About 75 ml distilled water was mixed with the sample in the digestive tube before distillation, and 25 ml boric acid as a receiver. The distilled solution was titrated with 0.2 M hydrochloric acid until the solution turned into a light pink colour. The crude protein content was determined using the following formula:

$$\text{Nitrogen content (\%)} = \frac{\text{Volume of HCl used (sample - blank)} \times N \text{ HCl} \times 14.007}{\text{Weight of sample (g)}} \times \frac{100}{1000}$$

$$\text{Protein content (\%)} = \text{Nitrogen content (\%)} \times 6.25 \quad (4)$$

where, 6.25 was the nitrogen conversion factor for all foods except cereal, legumes, milk, and milk products.

2.3.5 Carbohydrate Content

The total carbohydrate content was determined using the following calculation:

$$\text{Total carbohydrate (\%)} = 100\% - (\text{Moisture content (\%)} + \text{Total ash (\%)} + \text{Crude protein (\%)} + \text{Crude fat (\%)} \quad (5)$$

2.4 Total Sugar Content

The total sugar content was determined using high-performance liquid chromatography (HPLC) based on the method described by Wills *et al.* [16]. Sugar extraction was carried out using 85% boiled ethanol (25 ml) mixed with the sample (5 ml), and let it boiled in a waterbath for a few minutes. The solution was then filtered, and the extraction process was carried out three times before placing it in a rotary evaporator at 45 °C. The excess solution was placed in a 10 ml conical flask, and marked up with distilled water. The solution was ultra-filtered until 2 ml filtrate was produced before injected into HPLC (Waters, USA).

2.5 Analysis of Mineral Content

Mineral content was determined using Atomic Absorption Spectrophotometer (AAS) as in AOAC [15]. About 7 ml of hydrochloric acid was added to

the crucible contained ash, and placed on boiling water until dry. The ash was mixed with hot deionized water and filtered into a 100 ml conical flask, and marked up with deionized water. The stock solution was analysed using AAS, and determined using the formula:

$$\text{Mineral content} \left(\frac{\text{mg}}{100\text{g}} \right) = \text{Ppm} \times \frac{1}{1000} \times 100 \quad (6)$$

2.6 Antioxidant Analysis

2.6.1 Total Polyphenol Content

The polyphenol content of the juice was determined using Folic-Ciocalteu assay based on the method described by Zhang *et al.* [17] with a few modifications. About 20 µl sample and 100 µl Folin-Ciocalteu agents were mixed into 96-wellplate flat bottom. Samples were left for 5 minutes before adding 80 µl of 7.5% sodium carbonate, and kept in the dark at room temperature. After two hours, the absorbance was measured at 750 nm using a microplate reader (iMark, Bio-Rad, Canada). The results were expressed as mg Gallic acid equivalents (GAE)/100 ml.

2.6.2 DPPH Radical Scavenging Activity

DPPH assay was done based on the method used by Wang *et al.* [18] with a few modifications. About 100 µl of sample and 100 µl 0.3 mM DPPH were mixed in 96-wellplate flat bottom, and left for 30 minutes in the dark at room temperature before the absorbance was measured at 490 nm. The results were expressed as mM Trolox equivalence (TE)/ml.

2.6.3 Ferric Reducing Antioxidant Power (FRAP)

FRAP assay was done according to the method used by Benzie and Strain [19] with a few modifications. About 50 µl of product sample and 175 µl of warm FRAP reagent were mixed in 96-wellplate flat bottom, and incubated in the dark at room temperature for 5 minutes before the absorbance was measured at 595nm. The results were expressed as mM Trolox equivalence (TE)/ml.

2.7 Sensory Evaluation

There were 30 untrained panels involved in the sensory evaluation. Each panel was given two juice samples (J1: the newly developed concentrated juice, and J2: commercially available mixed juice) that were served in small plastic cups labelled with three-digit codes from a random number table. The attributes that were evaluated were colour, aroma, taste, concentration, sweetness, sourness, and overall acceptance. Sensory analysis on the juice was carried out using a 5-point hedonic scale (5=

extremely like; 1= extremely dislike) based on the method described by Abdullah [20].

2.8 Statistical Analysis

Data was analysed using SPSS version 20 (IBM Corporation, Armonk, New York, USA), and presented in mean \pm standard deviation from triplicate analysis of samples. Independent t-test was used to analyse the mean score difference of the attributes in the sensory evaluation between the newly developed concentrated juice and commercially available juice, significant at ($p < 0.05$).

3.0 RESULTS AND DISCUSSION

3.1 Proximate Proportion

From Table 1 it can be seen that the newly developed mixed concentrated juice mainly consisted of carbohydrate content (92.13%) with a small amount of protein (1.97%), and fat (0.39%) content. Pomegranate, date and honey are high in carbohydrate especially sugar [21, 22, 23]. The total sugar content for the mixed concentrated juice was 55.65 g/100 g and the most predominant sugar was fructose (25.33 g/100 g) followed by glucose (21.73 g/100 g), and sucrose (0.72 g/100 g). The low sucrose content may indicate that there is no added sugar in the juice except natural occurring sugar from the ingredients [23]. Previous studies had reported that the dominant sugar found in pomegranate, honey, and date were fructose and glucose [21, 22, 23]. Fructose also known as fruit sugar is the simplest form of carbohydrate (monosaccharide) that is sweeter than any other form of sugar, and it is found in abundance in honey, fruits, flowers, and root vegetables [24]. Moderate fructose consumption (< 50 g/day or $\sim 10\%$ of total energy intake) did not have any adverse effect on lipid, and glucose control while the intake < 100 g/day did not influence body weight in adults [25]. In addition, the intake of fructose (50 g) two hours before a meal could reduce appetite, and lipid intake [25]. About 100 ml of mixed concentrated juice provided 380 kcal which is about 19% of the average total calorie intake of 2000 kcal/day for adult Malaysian based on the Recommended Nutrient Intakes (RNI) for Malaysia (NCCFN 2017). The juice is quite concentrated as it only has 3.77% water content. Thus, the mixed juice can be consumed in two ways which is either by direct consumption, or by mixing it with water to reduce its concentration. The total ash content of the juice was 2.01% indicating that it has a high mineral content since the ash was from the residue of inorganic substances such as minerals that are available in the juice [27, 28].

Table 1 Nutrients content in the mixed concentrated juice

Parameters	Value
Moisture (%)	3.77 \pm 0.15
Total ash (%)	2.01 \pm 0.03
Protein (%)	1.97 \pm 0.31
Fat (%)	0.39 \pm 0.36
Total carbohydrate (%)	92.13 \pm 0.00
Total sugar (g/100 g)	55.65 \pm 1.30
Fructose (g/100 g)	25.33 \pm 0.49
Glucose (g/100 g)	21.73 \pm 0.43
Sucrose (g/100 g)	0.72 \pm 0.13
Maltose (g/100 g)	6.59 \pm 0.23
Lactose (g/100 g)	1.27 \pm 0.02
Calorie (kcal/100 g)	380 \pm 0.00

Note. Data is expressed as mean \pm standard deviation.

3.2 Mineral Content

The mixed concentrated juice is high in potassium (270.70 mg/100g), sodium (58.43 mg/100g), and magnesium (31.58 mg/100g) with small amounts of calcium (5.45 mg/100g), iron (1.07 mg/100g), and zinc (0.31 mg/100g) content as shown in Table 2. The recommended daily intake of potassium, sodium, magnesium, calcium, iron, and zinc based on RNI for healthy Malaysian adult are 4.7 g, 1.5 g, 420 mg, 1 g, 18 mg and 6.5 mg, respectively [26]. Minerals are micronutrients that are very important for the body to carry out its function properly. Potassium and sodium are needed to balance fluid in the body as well as for muscle contraction. Magnesium and zinc are needed to make protein and genetic materials, and to maintain a healthy immune system. Iron is used in the formation of red blood cells (haemoglobin), and also for energy metabolism. Potassium is the most abundant mineral found in pomegranate, date, and honey [29, 30, 31]. Pomegranate juice has been consumed worldwide, and it contains about 1283.30 mg/L of potassium, 107.53 mg/L of calcium, 96.02 mg/L of sodium, and 67.22 mg/L of magnesium [30]. The mineral content of the mixed juice is higher compared to the mineral content of pomegranate juice alone. This may be due to the mixture of three different ingredients in the newly developed juice. But date syrups obtained from various types of date had a higher potassium (460 to 860 mg/100 g), calcium (51 to 50 mg/100g), and magnesium (48 to 53 mg/100g) with lower sodium (0.6 to 1.0 mg/100g) content compared to the developed mixed juice which might be due to the types of date used [29]. The potassium, calcium, sodium and magnesium content in honey varied in range from $< \text{LOQ}$ to 448 mg/100g (LOQ = Limits of quantitation 10.55 mg/L for potassium), 1.12 to 35.2 mg/100g, 0.78 to 30.5 mg/100g, and 0.41 to 17.3 mg/100g, respectively, depending on the bee [31]. Maturity index, fruit variety, processing and treatment of samples highly affected nutrient content in the juice [32]. The high mineral content in the mixed concentrated juice can contribute to a person's total daily intake of minerals

especially potassium, sodium, magnesium, calcium, iron, and zinc.

Table 2 Mineral content in the mixed concentrated juice

Mineral	Value (mg/100 g)
Sodium	58.43 ± 0.41
Potassium	270.70 ± 1.25
Magnesium	31.58 ± 0.94
Calcium	5.45 ± 0.03
Iron	1.07 ± 0.11
Zinc	0.31 ± 0.01

Note. Data is expressed as mean ± standard deviation.

3.3 Total Polyphenol Content and Antioxidant Activity

In order to confirm final antioxidant activity result in food samples, The First International Congress on Antioxidant Method held in June 2004 has recommended the use of total polyphenol content test, and two other antioxidant methods [33]. DPPH antioxidant scavenging and Ferric Reducing Antioxidant Power (FRAP) are the methods that are always used to determine antioxidant activity in food samples. The principle of the DPPH method is to measure antioxidant reaction with an organic radical while the FRAP method measures antioxidant reaction with iron (III) complex [34]. Total polyphenol content can be categorised as low (<500 mg/100 ml), intermediate (500 to 2000 mg/100 ml), and high (>2000 mg/100 ml) [35]. The mixed concentrated juice contains a moderate amount of total polyphenol content (1752.10 mg/100 ml) with antioxidant activity of 12.80 mM/ml and 51.68 mM/ml based on DPPH, and FRAP assays, respectively (see Table 3). Currently, there is no established data on recommended daily intake of polyphenol and antioxidant. On average a person consumes a minimum of about 1 g of polyphenol from diet a day [36, 37, 38]. Although there is no exact amount of polyphenol which should be taken in a day, there is a study that associates the higher intake of polyphenol with longevity. According to Zamora-Ros *et al.* [39], the mortality rate is decreased among people who consume 650 mg/day of polyphenol compared to people who consume less than 500 mg/day [39]. Scalbert and Williamson [36] reported that the plasma concentration of polyphenol compound rarely exceeds 1 µM after consumption of 10 to 100 mg of single polyphenol compound, but the total plasma polyphenol concentration may be higher because of the existence of metabolites that are formed in body tissues, or by colonic microflora.

One of the advantages of consuming fruit juice pressed from whole fruit is that it will also contain polyphenol compound that is only present in the inedible part of the fruit [36]. Although total dietary antioxidant capacity did not influence the risk of developing cardiovascular disease, dietary polyphenol intake potentially lowers the risk of the disease [40]. The antioxidant activities of food were attributed to the polyphenol contents and

compositions of bioactive, and also non-polyphenol antioxidant [41]. The total polyphenol content of the newly developed mixed concentrated juice was higher compared to pomegranate concentrate (551.61 to 9695.17 mg/kg), date syrup (21.4 to 42 mg/100 g), and honey (10.3 to 98.0 mg/100 g) as reported in previous studies. This may indicate that the combination of the three ingredients could contribute to the higher polyphenol and antioxidant content in the mixed juice [31, 32, 42] Wootton-Beard *et al.* [40] reported that commercial juices mixed with a few types of fruits or vegetables showed higher polyphenol content and antioxidant capacity compared to a single fruit or vegetable juice.

The antioxidant activity of pomegranate juice reported by Mena *et al.* [44] using DPPH assay was a little bit higher (14.80 mM TE/ml), but lower using FRAP assay (17.90 mM TE/ml) compared to the newly developed mixed concentrated juice. Meanwhile, lower antioxidant activity was observed in dates sample, when using DPPH assay (0.3 to 5.1 mM TE/ g), but FRAP assay showed a higher value (28.20 to 275.00 mM TE/ g) compared to the mixed concentrated juice [45, 46]. As for honey, the DPPH (0.94-5.70 mM TE /g), and FRAP (4.27-6.67 mM Fe (II)/ g) values were lower compared to the mixed concentrated juice [47, 48]. The synergistic effect cannot be confirmed by just comparing previous studies since there are many factors that should be considered such as the origin of the fruits or ingredients, and the method used in the making of the juices or concentrate [49]. Extraction method, sample handling, and analysis could affect the phytochemical content in the juice thus affecting the polyphenol content and antioxidant activity value [50, 51, 52]. The high antioxidant capacity together with other nutritional value could make the newly developed mixed concentrated juice a positive addition to the diet.

Table 3 Antioxidant and polyphenol content in the mixed concentrate juice

Antioxidant Assay	Value
Total polyphenol content (mg GAE/100 ml)	1752.10 ± 200.90
DPPH (mM TE/ml)	12.80 ± 0.00
FRAP (mM TE/ml)	51.68 ± 0.51

Note. Data is expressed as mean ± standard deviation.

3.4 Sensory Evaluation

Table 4 shows the social-demographic data of the panels involved. Majority of the subjects (80%) were female and of Malay race (83.33%). Other races were Chinese (6.67%), Indians (4%), and others (3.33%). Most of the panels (83.33%) were in the age group of 20-29 years. The other subjects were aged between 30-39 years (10%), and ≥40 years (6.67%). This may be due to the sensory analysis was carried out in a campus where a majority of the students are in their twenties. The mean score for each attribute

assessed in sensory evaluation is shown in Table 5. The mixed concentrated juice can be considered acceptable among consumers as the mean score for attributes such as colour, aroma, taste, concentration, sweetness, sourness, and overall acceptance were 3.57, 3.47, 3.53, 3.63, 3.58, 3.23 and 3.57, respectively. There was no significant difference of the mean score between the newly developed mixed concentrated juice and commercially available mixed juice for all the attributes. This showed that the newly developed mixed concentrated juice was comparable with the commercial juice products already available in the market. Since taste preference differs between individuals, some people found the taste of the mixed concentrated juice not palatable due to its strong sourness or sweetness. There are many factors that could influence an individual's food acceptance which then affects the evaluation of food taste such as body state (thirst, hunger), learning and memory, psycho-social, cultural influences, and a variety of cognitive variables [53, 54]. Besides that, when products with multiple flavours were tested at the same time, the flavour of the first product tasted may affect the taste of the following products although the products design was the same which is known as hedonic contrast effect [55]. Based on the results of the sensory evaluation, the taste of the mixed concentrated juice can be further improved to meet consumer preferences, even though the results may not represent all age groups outside the campus community.

Table 4 Socio-demographic of subjects involved in the sensory evaluation

Variables	n=30 (%)
Gender	
Male	6 (20)
Female	24 (80)
Race	
Malay	25 (83.33)
Chinese	2 (6.67)
Indian	2 (6.67)
Others	1 (3.33)
Age	
20-29 years	25 (83.33)
30-39 years	3 (10)
≥ 40 years	2 (6.67)

Table 5 Mean score of sensory evaluation for the newly developed mixed concentrated juice (J1) and commercially available mixed juice (J2)

Hedonic criteria	Mean score	
	J1	J2
Colour	3.57± 0.73	3.30± 0.66
Aroma	3.47± 0.97	3.60± 0.50
Taste	3.53 ± 0.78	3.61 ± 1.10
Concentration	3.63 ± 1.03	3.32 ± 0.86
Sweetness	3.58± 0.72	3.43± 0.68
Sourness	3.23± 1.07	3.42± 0.62
Overall acceptance	3.57± 0.76	3.62± 0.86

Note. Data is expressed as mean ± standard deviation. No significant difference.

4.0 CONCLUSION

The newly developed mixed concentrated juice is nutritious, and provides a high source of energy, minerals and antioxidants. The mixed concentrated juice has the potential to be commercialised as a health juice which can be recommended to be consumed by healthy individuals as a supplement to increase the intake of minerals and antioxidants, especially polyphenol. However, it is not advisable for metabolic syndrome patients especially people with diabetic, and kidney problem due to the high content of sugar and minerals mainly potassium and sodium. Although the combination of more than one food could increase the nutrient, and antioxidant content of the foods, further studies on the synergistic effect needs to be done in the future.

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