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Research article

Effects of phenotype and wet milling procedures on the starch isolation

from sorghum (Sorghum bicolor L. Moench) grains

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Abstract: The current study tends to introduce the effects of three wet milling procedures and sorghum *(Sorghum bicolor L. Moench)* phenotype on starch recovery and some physico-chemical properties of starch isolated from grains. It explores the sorghum grains from landraces, cultivated in the Sahara of Algeria, which in fact has a high percentage of total starch with a little percentage of tannin compared to many regions of the world. This study attempts to unveil that the starch recovery, of fifteen starch isolates, ranged between 58.06% and 83.11%, and their total starch and protein contents ranged from 92.01% to 98.75% and 0.35% to 2.34% respectively. The extents kinetic curves of hydrolysis indicates that starch isolates have high susceptibilities for hydrolysis to glucose by glucoamylase from *Aspergillus niger*, and the degree of hydrolysis ranges from 50.85% to 81.45%. The results demonstrate that the wet milling procedures affect the starch recovery, and protein content and swelling power at 85 °C of starch isolates. The effect of grain phenotype appears in moisture content and swelling power at 95 °C.

Keywords: grain quality; starch isolation; physico-chemical properties; sorghum *(Sorghum bicolor L. Moench)* landraces

1. Introduction

Sorghum (*Sorghum bicolor L. Moench*) is one of the major sources of carbohydrates and proteins for people living in the semi-arid regions, and it is an important cereal that can be grown under cool climatic conditions [1]. In many developed countries, sorghum grain is used to produce all that feeds livestock (20.8 million tons), while in Asia; it is an important source for human nourishment (15.1 million tons). Quantities vary from one continent to another: Africa (8.0 million tons) and Central America (0.3 million tons) [2].

The higher level of starch content makes sorghum grains a potential source of commercial production via different procedures. The sorghum starch production by wet milling process involves chemical, biochemical, and mechanical operations to separate the grain into its main components and fractions: starch, protein, fiber, and lipids [3]. Sorghum grain as currently used as a potential renewable feedstock for production of fuels, ethanol, chemicals and other products to improve and maintain the economic development [4]. Also, sorghum can be exploited as an alternative starch source for diverse industrial applications: bakery, snacks, flavors and beverage clouds, canning, batters and breading, dressings, soups and sauces, confectionery and dairy products [5–7]. The yield, the chemical composition and physical properties of the starch isolates are affected by various factors, such as grain varieties, steeping treatments and steeping conditions [3,8]. In this context, several studies have demonstrated that the botanical origins and wet milling process conditions can have strong impacts on the starch hydrolysis [9].

Some regions in the Algerian Sahara are noted for having a wide diversity of sorghum landraces, actually grown in small spaces. The vegetative group saves all that animals would need for eight months of the year. To contribute to the value of the sorghum plants cultivation in Algeria, the laboratory research seeks to raise the efficiency of starch isolation from local grains. The concern of this study is to encourage industry to take advantage of using plant parts in food, energy and other sectors. Some earlier studies have shown that starches can be isolated with a high yield and purity from two sorghum cultivars by small-scales wet milling procedures with no effect on the morphological structure of starch granules [10,11]. Generally, the morphological of starch granules from sorghum landraces planted in Algeria are characterized by a big grain size ($36.0 \mu m$). They possess different forms; changing from polygonal, semi-spherical and semi-oval. They are additionally characterized by the presence of pores, channel, and indents on the granules [10,11]. The sorghum starches, classified as A-type diffraction pattern typical of cereal starches, has a higher viscosity value. It also presents higher temperatures of peak and lower gelatinization enthalpies [11].

The aim of this work is to evaluate the effect of plant phenotype and wet milling procedures on recovery, quality and some physico-chemical properties of starch isolates from grains of five sorghum landraces cultivated in Sahara of Algeria.

2. Materials and methods

2.1. Sorghum grains

Grains (SY106AS, SY108FE, SY208FE, SR106AS, SR107AS) from five predominant sorghum (*Sorghum bicolor L. Moench*) landraces are used as samples. They vary in color (three are yellow group and two are red group) and shape, grown under uniform field conditions at Tidikelt

region. This region is an important sorghum producer situated in the Algerian Sahara (Lat: 27.20429, Lng: 2.4878). The Tidikelt region includes a low annual rainfall rate (16.9 mm) and a high temperature in summer with a monthly rate of 45.2 °C. The World Reference Base (WRB) for Soil Resources classifies soil of this region as a solontchaks soil [12]. All samples are manually cleaned.

2.2. Physical properties of sorghum grains

The most important characteristics of this kind of grains were: 100-kernel and test weight were determined according to descriptors [13]. Grain dimensions were measured and calculated with the method described by Jain and Bal [14]. The clorox bleach test was used to determine the presence of pigmented testa by developed method of Waniska *et al.* [15]. Endosperm texture was evaluated subjectively through rating the proportion of corneous to floury endosperm on a scale of percentage of corneous, intermediate and floury sorghum grains, according to method described by Taylor and Taylor [16].

2.3. Chemical composition of sorghum grains

Some most important chemical compositions of sorghum grain were determined. The moisture content was determinated according the 44-15A approved procedures method [13]. The grain protein content was determined using micro-Kjeldahl method according to AACC method 46-13 using nitrogen conversion factor of 6.25 [17]. Total starch was determined by the enzymatic method described by Belhadi *et al.* [10] and Goni *et al.* [18] using an amyloglucosidase (300 U/mL, Sigma A-7255). The glucose concentration was determined using a glucose oxidase-peroxidase kit (ELITCH, France) and converted into starch content using a 0.9 factor. Absorbance was measured at 500 nm. The total phenols were determined using the Folin-Ciocalteu method [19]. The vanillin-HCL method was used to quantify condensed tannins [20].

2.4. Starch isolation procedures

Three different laboratory wet milling procedures (P1, P2 and P3) involving steeping, wet milling, filtration, centrifugation, separation and drying were used in this research.

2.4.1. Steeping conditions

100 g of grains from each sample were steeped at the following steeping conditions:

Conditions in P1: 200 mL of 0.25 % (w/v) NaOH aqueous solution at 8 °C for 24 h [1].

Conditions in P2: 200 mL of a solution prepared from distilled water and sodium metabisulfite (0.25 % sulfur dioxide) at 58 $^{\circ}$ C for 48 h [21].

Conditions in P3: 200 mL aqueous solution contained 0.25 % sulfur dioxide and 0.50 % (v/v) lactic acid at 58 °C, for 48 h [21].

2.4.2. Wet milling and separation

Fifteen samples of starch isolate were obtained from grains of landraces after pre-treatments through the three steeping conditions and wet milling separations. After steeping process, all grains

were washed with distilled water and wet milled using the procedure described by Beta *et al.* [1] and Belhadi *et al.* [10]. Sorghum grains were again washed and ground with an equal volume of water for 1 min at low speed, then for 4 min at high speed in a Waring blender. The slurry was filtered through a 125 μ m, 80 μ m and 50 μ m mesh sieve grains by analytical sieve shakers (AS 200 basic, Retsch). Material remaining on the sieve was rinsed with water (3 × 25 mL). Grinding and filtering processes were repeated on this material. After rinsing, material remaining on the sieve was discarded. Collected filtrate was allowed to stand for 1 hr. The filtrate was centrifuged at 760×g for (P1) and 5500×g for (P2 and P3), during 10 min by refrigerated centrifuge (Jouan E96) [10,22]. The gray-colored top protein layer was removed using a spatula. Excess water was added to suspend the sediment and was centrifuged during 3 min. Washing and centrifugation were repeated several times until the top starch layer became white. The starch fraction was dried for 24 h at 40 °C, milled in mortar and sieved in a 500 μ m mesh sieve [10,23].

2.5. Production yield and recovery of starch

The starch yield (Y%) and starch recovery (R%) were calculated by dry weight as follows (Equations 1 and 2) [10]:

$$R\% = \left(\frac{Y\%}{TS_g}\right) \times 100 \tag{1}$$

$$Y\% = \left(\frac{m_{s} \times (100 - H_{s}\%)}{m_{g} \times (100 - H_{g}\%)}\right) \times 100$$
(2)

Where H_g represents moisture of sorghum grain, H_s is the moisture of starch isolate, m_g is the sorghum grain mass in (g); m_s , represents the mass of starch fraction in (g), TS_g is the total starch in sorghum grain and starch isolate, respectively.

2.6. Physico-chemical properties of starch isolates

The moisture content was determined according to AACC methods 44-15A [17]. Starch and protein contents were determined by methods described in sorghum grain quality subsection. Iodine calorimetric method was used to determine the amylose content [1,10]. Swelling power (SP) and water solubility index (WSI) of starch isolates were determined at 55 °C, 65 °C, 75 °C, 85 °Cand 95 °C according to the method described by Li and Yeh [24]. WSI and SP were calculated as follows (Equations 3 and 4) [9,24]:

$$WSI = \left(\frac{W_1}{0.1}\right) \times 100\% \tag{3}$$

$$SP = \frac{W_s}{0.1(100\% - WSI)} (g/g)$$
(4)

Where W₁ represents dried supernatant weight; Ws represents sediment weighed.

2.7. Starch hydrolysis

The starch hydrolysis was determined according to method of souilah *et al.* [9]. Samples of starch (500 \pm 0.1 mg) were suspended in 25 mL acetate buffer solution with 4.5 pH followed by gelatinization in a water bath at 90 °C. The obtained suspension was shaken continuously at a speed of 1000 t/min and 55 °C. Glucoamylase 25 mL enzyme with a concentration of 0.01 U/mL was added. Aliquots were withdrawn at different times with a micropipette. Each time an aliquot was taken, the reaction was interrupted by adding 0.1 mL of trichloroacetic acid 50 % (w/v), and pH was adjusted to 7.0 by adding sodium bicarbonate powder. The extent of hydrolysis was measured from the amount of liberated glucose, which was determined by the glucose oxidase-peroxidase kit (ELITCH, France). The degree of hydrolysis (DH (%)) of each starch was calculated according to (Equation 5):

$$DH(\%) = \left(\frac{mg_{glucose} \times 0.9}{mg_{starch}}\right) \times 100$$
(5)

Where mg_{glucose} represents the weight of liberated glucose; mg_{starch} represents the weight of starch sample.

2.8. Statistical analysis

All analyses were measured in three replicates and expressed as mean. Statistical analyses were performed using the SPSS V.17.0 (SPSS software, Inc., Chicago, IL, USA) for windows using Duncan method. Analysis of variance (ANOVA) and hierarchical cluster analysis (HCA) methods were performed to compare starch isolates properties; differences were considered at the significant level of 95% ($p \le 0.05$). Graphs were created using Sigma Plot (V.10.0 Systat Software Inc, Wpcubed GmbH, Germany) for Windows.

3. Results and discussion

3.1. Physical properties

Table 1 shows the results of the physical (quantitative and qualitative) parameters and the most important chemical compositions of sorghum grains. Of the five sorghum phenotypes, three grains: (SY106AS, SY108FE and SY208FE) are classified in yellow group and two (SR106AS and SR107AS) are arranged in class of red group. All grains were classified with a thick pericarp (Figure 1). The thick pericarp is much easier to peel; this is one of the reasons why in some African countries' farmers prefer to grow this type of sorghum grains [25]. When treating the grains with chlorox bleach, the result shows that sorghum grains are without a pigmented testa. The grains are characterized by low moisture content (<12%), and can be stored without rot which leads to an increase in storage life [26]. The grains have similar size and shape. The grain is generally a flattened sphere approximately 4.86 mm long by 4.27 mm wide and 2.65 mm thick (Figure 1). These means are slightly higher than those of American commercial sorghum grain [27], while they are similar to those which are grown in Kenya [28]. According to Gomez *et al.* [26], the grains are suitable for milling, because the weight of 100 kernels is superior to 2 g. The mean of test weight is 707.96 g/L,

it is integrated in the values of the African sorghum grain [27] however; it is lower than those of the American commercial sorghum grain [27,29]. The examination of endosperm texture shows a variances in percentage of corneous, intermediate, and starchy fractions, this result indicates much variations in endosperm texture and should be classified as mixed endosperm texture [30].



Starchy endosperm

Intermediate endosperm

Corneous endosperm



3.2. Chemical composition of sorghum grain

The means of chemical compositions of grain are (Table 1): starch, 68.65%; protein, 13.25%; phenol, 2.33 mg/g; tannin, 2.54 mg/g in dry matter. When comparing the results of the five sorghum landraces to those of the world collection at ICRISAT, it must be pointed out that the mean of starch content is slightly lower than the mean value (69.50%), whilst the protein content is higher than the mean value (11.40%) [5].

The means of phenol content of the yellow and red phenotype are 0.54 mg/g and 5.02 mg/g, respectively. These means are lower than those given by Awika and Rooney (0.80 mg/g from yellow grain and 6.60 mg/g from red grain) [31]. Also, Awika and Rooney have classified the tannin-free sorghum grain when the values up to 4.00 mg/g [31]. These results agree that the sorghum grains of Tidikelt region do not contain tannins except the red phenotype SR106AS which is likely to contain tannin. Moreover, the results demonstrate that SY106AS, SY108FE, SY208FE and SR107AS phenotype can be classified by the type I (sorghum tannin-free) and the red SR106AS phenotype is taking the weak values of the type II [32].

Sorghum landraces		SY106AS	SY108FE	SY208FE	SR106AS	SR107AS
Grain color		Yellow	Yellow	Yellow	Red	Red
Munsell chart		5GY9/4	5GY9/4	5GY9/6	10YR6/10	7.5YR7/12
Moisture (%)		11.25	10.65	09.66	11.39	08.44
Test weight (g/L)		714.18	702.34	707.76	718.44	697.09
Starch (%, db)		66.30	70.41	67.33	77.20	62.00
Protein (%, db)		11.17	13.20	13.44	14.68	13.75
Phenol content (mg/g)		0.51	0.63	0.49	5.95	4.09
Tannin content (mg/g)		2.01	0.00	0.00	7.58	3.12
100-kernel weight (g)		3.34	3.43	3.30	2.89	3.51
L (mm)		4.71	4.82	4.80	4.83	5.13
W (mm)		4.15	4.36	4.33	4.037	4.46
T (mm)		2.70	2.71	2.82	2.45	2.59
Endosperm	Int	5.00	15.00	35.00	70.00	70.00
texture (%)	Sta	0.00	0.00	0.00	10.00	5.00
	Cor	95.00	85.00	65.00	20.00	25.00

Table 1. Grain quality evaluation: qualitative, quantitative parameters and chemical compositions of sorghum grain landraces.

Note. db: dry basis; L: length of sorghum grain; W: width of sorghum Grain. T: thickness of sorghum grain; Int: Intermediate; Sta: Starchy; Cor: Corneous.

From the short review above, key findings emerge: the sorghum grains grown in the Tidikelt region have a high percentage of starch and protein. The possession of some physical (quantitative and qualitative) parameters and most important chemical compositions indicates that those grains can be used in the milling applications such as, production of meals, flours and grits [26]. In general, these grains are differing from other grains grown in many parts of the world, because they are classified in sorghums without a pigmented testa, and they do not contain large percentages of the tannin compounds, although they are rich in phenolic compounds. We speculate that this might be due to the influence of environmental factors [27]. Environmental conditions and genetic factors can vary significantly the sorghum grain structure, quality and nutritional importance [33,34].

3.3. Recoveries, chemical and physicochemical properties of starch isolates

The starch recovery was ranging between 58.06% and 83.11%. Here we compared the results between the proposed procedures, recovery of the (P3) was higher (69.85%–83.11%) than those obtained by (P2) (68.39%–78.37%), and then those from (P1) (58.06%–70.60%). When comparing our results to those of previous studies from sorghum wet milling, it must be pointed out that the highest starch recovery obtained were similar to results found by Buffo *et al.* (71.37%–9.71%) [29] using (P3), and by Xie and Seib (83.70%) [35] and Wang *et al.* (85.90%) [36] using (P2), while it was lower than those obtained by Higiro *et al* (96.5%) [37] who used (P3). Starch yield (50.99%) was similar to that obtained by Yang and Seib (50.50%) with (P2) [22].

The contents of starch isolates evaluated by; total starch analysis was ranging between 92.01% and 98.75%. Moisture content ranged from 10.96% to 12.95%. Amylose content ranged between 23.78% and 27.74%, which indicated that sorghum samples were non-waxy starch with normal amylose

content. Protein content gave an indication of the degree of separation and purity [3], while ranging between 0.35% and 2.34%. This analysis found evidence for starches obtained by (P1) (ranging between 0.35 and 0.77%) was the lowest protein content than from the (P2) (from 1.07% to 1.94%), and the (P3) (from 1.54% to 2.34%), respectively. The same results have been reported by Belhadi *et al.* [10], Xie and Sieb [35] and Wang *et al.* [36] when they used the (P1).

Water solubility index (WSI) and swelling power (SP) showed a non-linear increase with temperature. Figure 2 (A), (C) and (E) showed that the swelling power increased with increasing temperatures. At temperatures range between 75 °C and 85 °C, we observed a very slow increase of swelling power for majority of starch isolates. When, the temperature higher than 85 °C, the swelling power of all starches had started over to swell. By comparing the results of all cases, starches from (P1) had the highest swelling power ranging from 3.41 g/g to 38.83 g/g, followed by (P2) from 3.37 g/g to 34.25 g/g and these lasts were higher than that of (P3) (from 3.19 g/g to 33.12 g/g). Overall, these findings were in accordance with findings reported by Wang and Seib [38], showing that the swelling power of starches from (P3) was slightly different from (P2), and it might be the effect of the addition of lactic acid or protein content, which could inhibit the swelling of starch granules, similar results were obtained by Shandera and Jackson [39] and Brandemarte *et al.* [40].

The water solubility index of starch isolates has illustrated similar trend, increasing with increasing temperature, it ranged between 0.40% and 38.60% Figure 2 (B), (D) and (F). When comparing among the three procedures, the results showed that the water solubility index of starch isolates obtained by (P1) were higher than that of (P2) and (P3). Whilst, the less soluble starch was observed on starches isolated from SY108FE and SY208FE sorghum grains. Those results suggested a relationship between the solubility of isolated starch and the endosperm texture of sorghum grains. The intermediate (SR106AS and SR107AS) and the corneous (SY106AS) endosperm texture had a more high water solubility index. However, Bello *et al.* [41] found more starch solubilization corresponded to the more corneous endosperm, when using the sorghum flour during the preparation of tô.

3.4. Sorghum starches hydrolysis

Figure 3 presents the kinetic curves of sorghum starches hydrolysis by glucoamylase from *Aspergillus niger*. The extents of the reaction indicated that these starches had high susceptibilities for hydrolysis to glucose.

Among sorghum starches, the means degree of hydrolysis followed this order: (P1) (71.41%) > (P3) (67.75%) > (P2) (67.09%). Planned comparisons revealed that the decrease in starch hydrolysis while adding lactic acid was confirmed by Brandemarte *et al.* [40], except for SY208FE starch isolate its addition leads to an increase in starch hydrolysis Figure 3 (C). Extensive results carried out showed that, the differences in the in vitro digestibility of starches have been attributed to the interplay of many factors such as steeping conditions [9] and botanical starch source [42].



Figure 2. Swelling power and water solubility index of starch from sorghum grain isolated by Procedure 1 (A) and (B), Procedure 2 (C) and (D) and Procedure 3 (E) and (F).

3.5. Results of statistical analysis

A statistical analysis was performed by using the ANOVA applying a significance level of $p \le 0.05$. The Tables 2 and 3 shows the means of starch recoveries, components and physicochemical properties of starch isolates from five sorghum grain phenotypes (SY106AS, SY108FE, SY208FE, SR106AS and SR107AS) isolated by three wet milling procedures (P1, P2 or P3).

The evaluation of the data presented in this work leads to analyze two different points of view such as; the effect of grain phenotype and wet milling procedures on starch recovery, components and some physico-chemical properties of starch isolate. First, the effect of wet milling procedures indicated that there were only three significant differences that are represented in starch recovery, protein contents and swelling power at 85 °C (Table 2).



Figure 3. The progress curves of sorghum starches hydrolysis by glucoamylase from *spergillus niger*, at S₀=10 g/L, extracted from (A: SY106AS, B: SY108FE, C: SY208FE, D: SR106AS and E: SR107AS) by all treatments, at a 0.01 U/mL concentration of the enzyme, pH = 4.5 and 55 °C.

Table 2. Starch recovery, chemical, and physicochemical properties of fifteen starch isolated from five sorghum grain samples isolated by three procedures: P1, P2, and P3.

Starch isolation procedures	Procedure (P1)	Procedure (P2)	Procedure (P3)
Starch recovery (%)	63.33 ^a	74.41 ^b	78.08 ^b
Moisture (%)	12.00 ^a	11.57 ^a	11.68 ^a
Total starch (%)	96.81 ^a	96.38 ^a	95.04 ^a
Protein content (%)	0.58 ^a	1.51 ^b	1.96 ^c
Amylose (%)	25.48 ^a	25.12 ^a	24.96 ^a
Swellig power (85 °C; g/g)	16.85 ^b	14.02 ^a	14.04 ^a
Degree of hydrolysis (%)	71.41 ^a	67.75 ^a	67.09 ^a

Note. ANOVA followed by Duncan test was performed to search for properties differences in the samples. In a row, means followed by the same letters (a, b or c) are not significantly different ($p \le 0.05$) between procedures.

Sorghum grain samples	SY106AS	SY108FE	SY208FE	SR106AS	SR107AS
Starch recovery (%)	72.57 ^a	77.36 ^a	71.48 ^a	65.43 ^a	71.94 ^a
Moisture (%)	11.26 ^a	11.68 ^a	11.25 ^a	11.92 ^a	12.65 ^b
Total starch (%)	95.12 ^a	95.41 ^a	96.46 ^a	96.80 ^a	96.60 ^a
Protein content (%)	1.46 ^a	1.21 ^a	1.66 ^a	1.33 ^a	1.10 ^a
Amylose (%)	25.26 ^a	24.84 ^a	25.03 ^a	24.72^{a}	26.09 ^a
Swellig power (95 °C; g/g)	26.88 ^a	28.08 ^{a,b}	26.67^{a}	34.06 ^b	33.74 ^b
Degree of hydrolysis (%)	66.28 ^a	62.19 ^a	69.03 ^a	70.62 ^a	75.63 ^a

Table 3. Starch recovery, chemical, and physicochemical properties of fifteen starch isolated by three procedures for the five sorghum grain samples.

Note. ANOVA followed by Duncan test was performed to search for properties differences in the samples. In a row, means followed by the same letters (a or b) are not significantly different ($p \le 0.05$) between starch isolates from sorghum grain samples.





Second, the analysis by different grain phenotype, ANOVA indicated that there were only two significant differences that are represented in moisture content and swelling power at 95 °C (Table 3).

Hierarchical cluster analysis (HCA) identified the similarity groups among all starch fractions of this study. The dendrogram divided the fifteen starches into four main clusters, classified by the phenotype of grains and by the wet milling procedure methods (Figure 4). The first main cluster was produced at a distance of 4.5 and included three samples from red grains phenotype; two samples from SR107AS were isolated by (P1) and (P2) and the third was isolated by (P1) from SR106AS. The second main cluster was formed at a distance of 3.5 and comprised three samples of yellow grains phenotype were isolated by (P1) from SY106AS, SY108FE and SY208FE. The third and

fourth clusters were formed at a distance of 6.5. This clusters consisted of nine starch samples isolated by (P2) and (P3) from red and yellow sorghum grain landraces.

4. Conclusions

The results demonstrate that there is a strong effect of wet milling procedures on starch recovery, protein contents and swelling power at 85 °C of starch isolate. While the effect of grain phenotype appears in moisture content and swelling power at 95 °C. Moreover, the ANOVA find no significant differences in the other properties at a significant level $p \le 0.05$.

On this basis, we conclude that the Algerian Sahara has a good sorghum landrace with a high grain quality and chemical compositions such as test weight, 100-kernel weight, endosperm texture, moisture, amylose, swelling power, water solubility index, starch, protein content and degree of hydrolysis. On the other hand, the isolation of sorghum starch has a respectable recovery (up to 83.11%) with a good purity accompanied by a high percentage of starch content up to 98.75% and a low percentage of protein content up even 0.35%. These findings consolidate that the Algerian sorghum grain can play an important role in the food and nonfood applications, and encouraging the uses of industrial wet milling methods. The interpretation of the results has shown the effects of the wet milling procedures and sorghum grain phenotype on starch isolation.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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