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Kinetics of Hydrolysis of Fructooligosaccharides in Mineral-Buffered Aqueous Solutions: Influence of pH and Temperature

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High-performance anion exchange chromatography coupled with a pulsed amperometric detection system (HPAEC−PAD) was used to evaluate the extent of chemical hydrolysis of three fructooligosaccharides (FOS) including 1-kestose (β-D-Fru-(2→1)$_2$-α-D-glucopyranoside, GF2), nystose (β-D-Fru-(2→1)$_3$-α-D-glucopyranoside, GF3), and fructofuranosylnystose (β-D-Fru-(2→1)$_4$-α-D-glucopyranoside, GF4). A kinetic study was carried out at 80, 90, 100, 110, and 120 °C in aqueous solutions buffered at pH values of 4.0, 7.0, and 9.0. Under each experimental condition, the determination of the respective amounts of reactants and hydrolysis products showed that FOS hydrolysis obeyed pseudo-first-order kinetics as the extent of hydrolysis, which decreased at increasing pH values, increased with temperature. The three oligomers were found to be degraded mainly under acidic conditions, and at the highest temperature value (120 °C), a quick and complete acid degradation of each FOS was observed. Using the Arrhenius equation, rate constants, half-life values, and activation energies were calculated and compared with those obtained from sucrose under the same experimental conditions. It appeared that the hydrolysis of FOS took place much more easily at acidic pH than at neutral or basic pH values.

KEYWORDS: Fructooligosaccharides; high-performance anion exchange chromatography; pulsed amperometric detection; hydrolysis; pH; temperature; kinetics

INTRODUCTION

Fructooligosaccharide (FOS) is the common name for fructose oligomers of which the three major representatives are usually known as 1-kestose (β-D-Fru-(2→1)$_2$-α-D-glucopyranoside, GF2), nystose (β-D-Fru-(2→1)$_3$-α-D-glucopyranoside, GF3), and fructofuranosylnystose (β-D-Fru-(2→1)$_4$-α-D-glucopyranoside, GF4). In all of them, fructosyl units (F) are bound to the β (2→1) position of sucrose (GF) (*1*).

They are widely distributed throughout the plant kingdom, in fruits including banana and plum, as well as in vegetables including onion, shallot, chicory, and artichoke (*2*, *3*). FOS can also be produced on a commercial scale from sucrose using a fungal enzyme from either *Aureobasidium sp* (*4*, *5*) or *Aspergillus niger* (*6*). In addition to the fact that the way they are enzymaticaly produced is accurate and that their sweet taste is quite comparable to that of sucrose, the traditional sweetener, their low caloric content is worth stressing.

FOS are not absorbed in the human small intestine but readily fermented by the colonic flora. They promote selectively the growth of bifidobacteria in the colon, which are considered to be beneficial to human health (*6*−*8*). Their increasing interest as a prebiotic, components able to stimulate specifically in humans and other mammals the growth and/or the activity of a restricted number of commensal bacterial species, is also strengthened by both their low glycemic and lipogenic effects (*9*−*12*).

FOS are considered as alternative sweeteners which are increasingly consumed on account of their nutraceutical value as health and reduced caloric content food. The above-mentioned GF2 and GF3 products as well as other GF*n* units of nutritional interest are used as food ingredients (*13*) in a variety of foods, namely in bakery (chocolate, biscuits, pastries) and dairy products, and are commercially available as analytical standards (Wako Pure Chemical Industries, Osaka, Japan).

In a previous paper (*14*), we were able to show the suitability of high-performance chromatography using a CarboPac PA 100 anion-exchange column and the new quadruple-potential waveform to separate and quantify in a single run the following fructans inulobiose (F2), 1-kestose (GF2), and nystose (GF3) present in a food matrix such as fresh fruit and stewed fruit obtained from a local manufacturer. Furthermore, a kinetic study was already performed to determine the dependence of the acid-hydrolytic rate-constants on the degree of oligosaccharides polymerization in juices of the Jerusalem artichoke (*15*). The

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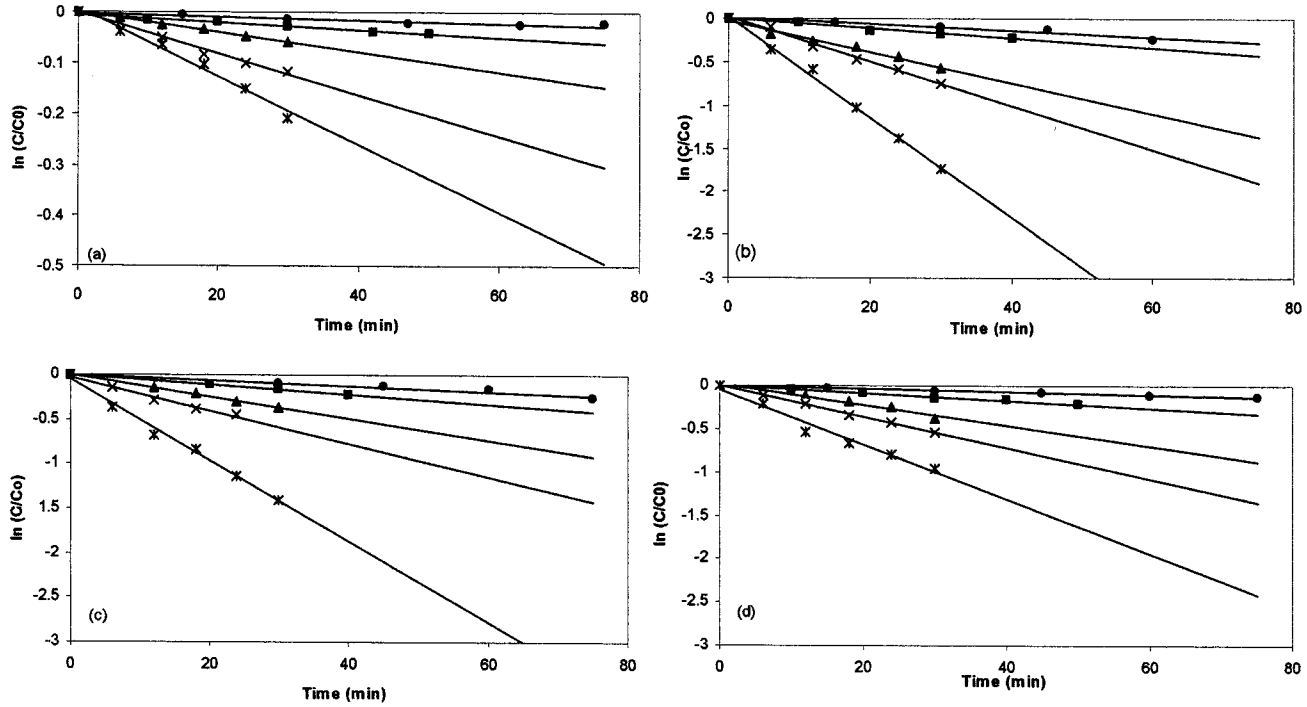

Figure 1. Kinetics of hydrolysis of a number of oligosaccharides at pH 4.0: sucrose (a), 1-kestose (b), nystose (c), and fructofuranosylnystose (d) at 80 °C (●), 90 °C (■), 100 °C (▲), 110 °C (×), and 120 °C (*).

Table 1. Buffered Aqueous Solutions Used to Carry out FOS Hydrolysis

pH	adjusted to 100 mL of final volume with water
4.0	30.7 mL of 0.2 M citric acid + 19.3 mL of 0.2 M Na_2HPO_4
7.0	21 mL of 0.2 M NaH_2PO_4 + 29 mL of 0.2 M Na_2HPO_4
9.0	50 mL of 0.025 M $Na_2B_4O_7 \cdot H_2O$ + 4.6 mL of 0.1 M HCl

aim of the present study was to determine the influence of pH and temperature on the rate of sucrose and FOS hydrolysis, as well as to put forward the mechanism whereby this reaction takes place in order to evaluate the consequences of heating food products on their FOS content.

MATERIALS AND METHODS

Chemicals. Standard sucrose, D-glucose, and D-fructose were purchased from Sigma (St Quentin Fallavier, France), while the standard fructooligosaccharide set containing GF2, GF3, and GF4 was from Wako (Neuss, Germany) with 99%, 99%, and 98% purity, respectively. Sodium tetraborate decahydrate (borax, ACS grade), sodium acetate (no. 1.06268), and citric acid monohydrate (no. 1.00244) were purchased from Merck (Nogent-sur Marne, France). Sodium hydroxide of 46−48% (w/w) aqueous solution (S/4930) was from Fisher Scientific (Elancourt, France). Ultrapure 18 Ω cm deionized water used in the experiments, including that used as HPAEC−PAD solvent, was obtained from a Maxima ultrapure water system (Elga, Decines, France). Sodium dihydrogenophosphate dihydrate and hydrochloric acid (minimum 36%) were obtained from Prolabo (Fontenay Sous-bois, France).

FOS and Sucrose Degradation in Aqueous Solutions. Aqueous-buffered solutions (50 μM) of pure sucrose (GF), 1-kestose (GF2), nystose (GF3), and fructofuranosylnystose (GF4) at pH 4.0, 7.0, and 9.0 were prepared as indicated in **Table 1** using ultrapure deionized water. Each oligosaccharide solution (1 mL) at a given pH value was incubated in a thermostated paraffin bath under five different temperature conditions, namely 80 ± 2, 90 ± 2, 100 ± 2, 110 ± 2, and 120 ± 2 °C. After aliquots were sampled at appropriate time intervals and hydrolysis was stopped by cooling in an ice bath, they were successively passed through a 0.2 μm cutoff microfilter, 2-fold diluted with water, and analyzed by HPAEC−PAD.

Analysis of Hydrolysis Products by HPAEC−PAD. This was achieved with a Dionex system DX500 (Sunnyvale, CA) consisting of a GP 50 gradient pump with on-line degassing and an ED40 electrochemical detector, operating in the PAD mode as previously described (*14*). Quantitative determination of the reaction products (glucose, fructose, sucrose, 1-kestose, and nystose) was carried out using a CarboPac PA100 (Dionex) analytical anion-exchange column (250 × 4 mm) equipped with a guard column CarboPac PA100 (50 × 4 mm). The elution was performed at a constant flow rate of 1 mL/min and at room temperature, using a 20-min linear gradient from 0% to 40% of a 80 mM NaOH−500 mM sodium acetate in 80 mM NaOH−5 mM sodium acetate. Both eluents were prepared with ultrapure water and degassed by nitrogen bubbling. The injection volume was 20 μL, and each analysis was done in triplicate.

RESULTS AND DISCUSSION

Effect of pH on Sucrose and FOS Hydrolysis. The influence of both pH and temperature on the chemical hydrolysis of 1-kestose (GF2), nystose (GF3), and fructofuranosylnystose (GF4) in buffered and sterile aqueous solutions was studied at five temperatures ranging from 80 to 120 °C and at three pH values (4.0, 7.0, 9.0). The decrease in GF, GF2, GF3, and GF4 concentrations at pH 4.0 is shown in **Figure 1 a−d**, respectively. The $\ln(C/C_0)$ versus time plot of sucrose and the three FOS tested gave straight lines at each temperature value, indicating that hydrolysis followed first- or peudo-first-order kinetics (*16*, *17*):

$$C = C_0 \exp(-k_{obsd} t) \quad (1)$$

Where t is the incubation time in minutes, C is the concentration of sucrose or FOS in mol·L^{-1} at time t, C_0 is the initial concentration in mol·L^{-1}, and k_{obsd} in min^{-1} is the observed rate constant of the reaction. Similar results were obtained when sucrose and FOS were incubated at pH 7.0 (data not shown).

Table 2 shows the hydrolysis rate constant (k_{obsd}) and half-life ($t_{1/2} = \ln 0.5/k_{obsd}$) values obtained for sucrose, 1-kestose, nystose, and fructofuranosylnystose at pH 4.0 and increasing

Table 2. Hydrolysis Rate Constant (k_{obsd}) and Half-Lives ($t_{1/2}$) at pH 4.0 and pH 7.0 and Different Temperatures

		GF		GF2		GF3		GF4	
pH	T (°C)	k_{obsd} (min^{-1})	$t_{1/2}$ (min)	k_{obsd} (min^{-1})	$t_{1/2}$ (min)	k_{obsd} (min^{-1})	$t_{1/2}$ (min)	k_{obsd} (min^{-1})	$t_{1/2}$ (min)
4.0	80	3×10^{-4}	2310	37×10^{-4}	187	32×10^{-4}	217	17×10^{-4}	408
	90	8×10^{-4}	866	56×10^{-4}	124	54×10^{-4}	128	42×10^{-4}	165
	100	20×10^{-4}	346	177×10^{-4}	39	122×10^{-4}	57	119×10^{-4}	58
	110	40×10^{-4}	173	254×10^{-4}	27	187×10^{-4}	37	178×10^{-4}	39
	120	67×10^{-4}	103	580×10^{-4}	12	472×10^{-4}	15	315×10^{-4}	22
7.0	80	6×10^{-6}	115524	4×10^{-5}	17329	7×10^{-5}	9902	11×10^{-5}	6178
	90	29×10^{-6}	23656	7×10^{-5}	9902	12×10^{-5}	5579	15×10^{-5}	4593
	100	37×10^{-6}	18683	22×10^{-5}	3068	24×10^{-5}	2944	27×10^{-5}	2538
	110	200×10^{-6}	3465	30×10^{-5}	2309	31×10^{-5}	2221	60×10^{-5}	1137
	120	656×10^{-6}	1056	50×10^{-5}	1374	62×10^{-5}	1119	74×10^{-5}	939

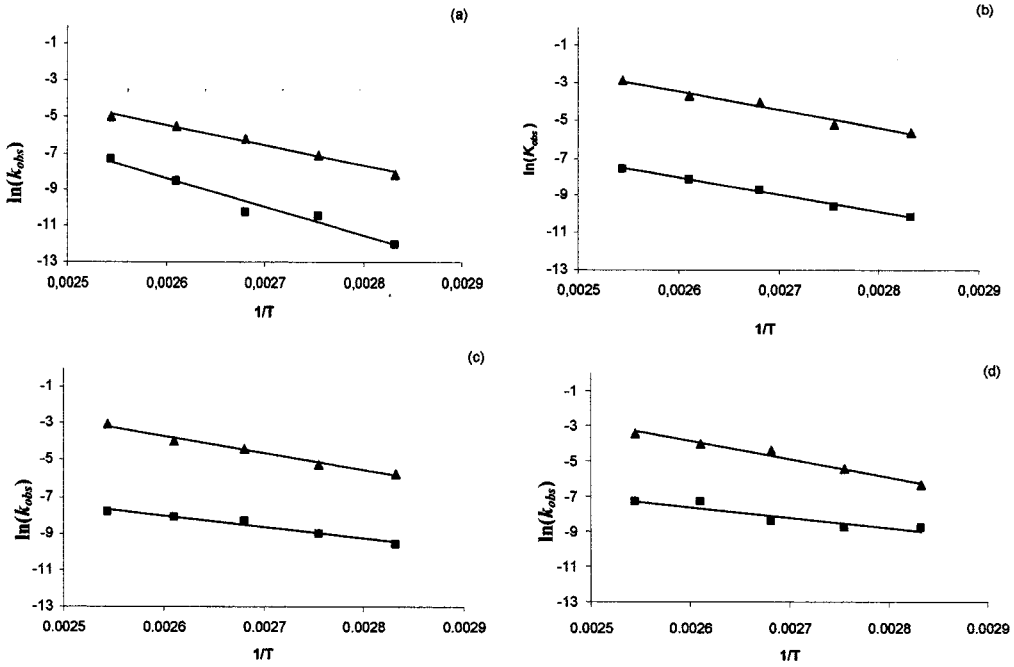

Figure 2. Arrhenius plots of FOS hydrolysis at pH 4.0 (▲) and pH 7.0 (■): sucrose (a), 1-kestose (b), nystose (c), and fructofuranosylnystose (d).

temperatures from 80 to 120 °C. A 40-degree increase in the incubation temperature at pH 4.0 resulted in comparable increases in the observed hydrolysis rate constants of sucrose and FOS (22-fold and 15- to 19-fold, respectively), although the stability of the former was higher than that of the latter ($t_{1/2}$: 103 min and 12−22 min, respectively). At the highest temperature (120 °C), the three FOS were rapidly degraded although the higher the number of fructose units, the higher the stability as indicated by the half-life values of 1-kestose (GF2), nystose (GF3), and fructofuranosylnystose (GF4), which were found to be of 12, 15, and 22 min, respectively. At pH 7.0, the stability of sucrose as well as that of FOS were found to be greatly enhanced whatever the incubation temperature. Sucrose seemed to be the stablest except at the highest temperature under which GF2 and GF3 were found to be the stablest. No degradation of sucrose and FOS was observed after 24 h of incubation at pH 9.0, in the 80−120 °C temperature range (data not shown).

Effect of Temperature on Sucrose and FOS Hydrolysis. The effect of temperature on the rate of sucrose and FOS hydrolysis was determined using the Arrhenius law:

$$k_{obsd} = A \exp[-E_a/(RT)]$$

Where A is a specific constant for the reaction, E_a is the activation energy in J·mol^{-1}, R is the universal gas constant (8.314 J·mol^{-1}·K^{-1}), and T is the absolute temperature in K.

Table 3. Arrhenius Equation, Coefficient of Determination (r^2), Activation Energy (E_a), and Frequency Factor (A) at pH 4.0 and pH 7.0

pH	compd	$\ln k = f(1/T)$	r^2	E_a (kJ·mol^{-1})	A (min^{-1})
4.0	GF	$-10887(1/T) + 22.834$	0.9912	90.5	82×10^{8}
	GF2	$-9726(1/T) + 21.846$	0.9753	80.9	31×10^{8}
	GF3	$-9169(1/T) + 20.138$	0.9841	76.2	55×10^{7}
	GF4	$-10146(1/T) + 22.480$	0.9795	84.6	58×10^{8}
7.0	GF	$-15657(1/T) + 32.335$	0.9640	130.2	11×10^{13}
	GF2	$-9060(1/T) + 15.515$	0.9922	75.4	55×10^{5}
	GF3	$-7327(1/T) + 11.199$	0.9888	60.9	7×10^{4}
	GF4	$-6812(1/T) + 10.121$	0.9328	56.7	2×10^{4}

Figure 2 shows the Arrhenius plots [$\ln k_{obsd} = f(1/T)$] at pH 4.0 and pH 7.0 from which the E_a values were calculated. The frequency factor (A) represents the value of k_{obsd} at infinite temperature, which means that all the reactants have the required activation energy for reacting or the probability to react.

Table 3 gives the activation energy (E_a) and frequency factor (A) values of FOS hydrolysis at pH 4.0 and pH 7.0. The E_a values for GF2, GF3, and GF4 were found to be lower than that of GF irrespective of pH. By contrast, these values are higher at pH 4.0 than at pH 7.0 for FOS, while in the case of sucrose it is the opposite. The high stability of FOS at pH 9.0 and low stability at pH 4.0 is consistent with the involvement of protons in the degradation process and thus with a catalytic

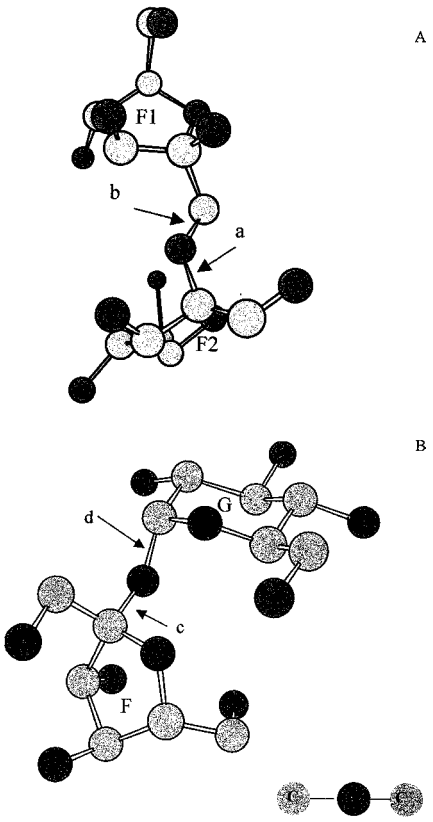

Figure 3. Molecular models of inulobiose F1–F2 (A) and sucrose F–G (B) osidic bonds tested, indicated as a–d, respectively.

acid mechanism. Our results are in agreement with the average activation energy (109 ± 10 kJ/mol) obtained for five commercially available mixes of oligofructose samples (Actilight 950P, Raftilose P95, Fibrulose 97, Fibruline instant, and Fibruline Long Chain) incubated in an acidic media (*18*). Nevertheless, this study was undertaken with mixtures while our work have been realized with pure products. Furthermore, they analyzed the fructose formation, since we followed directly the disparition of the oligosaccharides.

The higher stability observed under neutral or basic condition, and consequently lower stability at acid pH, could therefore be associated with a protonic activation of the leaving group. To try to put forward the fragility of the C−O osidic bond when oxygen is protonated, an elongation of the bond was simulated up to the breaking value, using the quantum semi-empirical mechanical molecular model technique AM1 (*19*, *20*) and according to the method of the reactional path. The solid geometry of the most stable configuration of sucrose and inulobiose was progressively modified by imposing variable lengths to the two C−O osidic bonds surrounding the glycosidic oxygen atom, and each time the molecular geometry was adjusted to lose the minimal energy value. As shown in **Figure 3**, the selected bonds in inulobiose are indicated as a and b and those in sucrose as c and d in each non-protonated form, while they were indicated as a1, b1, c1, and d1 in the corresponding protonated forms in which a proton is linked to the glycosidic oxygen atom. **Figure 4** clearly shows that the fragility of the two bonds involved in the glycosidic linkage in both protonated inulobiose and sucrose was significantly higher than those in the non-protonated forms. Thus, the two protonated oligosaccharides are more rapidly hydrolyzed at acid pH than at neutral or basic pH values. This kind of analysis is in conformity with the principle of "non crossing rule" (*21*).

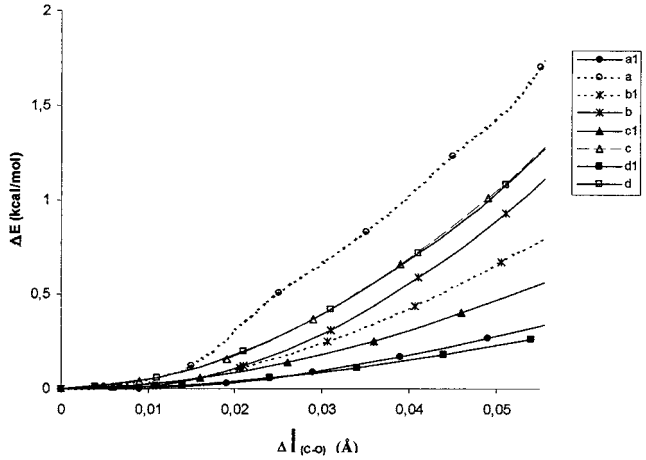

Figure 4. Bond fragility of both protonated inulobiose (a1, b1) and sucrose (c1, d1) versus the nonprotonated form (a–d, respectively). Variation of the energy of formation (*E*) depends on the length of osidic bond C–O.

CONCLUSION

The experiments on fructooligosaccharide degradation in aqueous media, which were carried out over a wide range of temperatures at three different pH values, showed that the hydrolysis reaction of 1-kestose (GF2), nystose (GF3), and fructofuranosylnystose (GF4) followed pseudo-first-order kinetics. Furthermore, it appeared that the hydrolysis of FOS took place mainly at acidic pH rather than at neutral or basic pH values. It is well-known that sucrose degrades faster at lower pH and higher temperatures (*22*). Concentration has also a very large influence on the degradation of sucrose. Only one concentration was studied here, which was very low, and the water activity would be extremely high. Often in foodstuffs, where fructooligosaccharides and sucrose are added as ingredients, the water activity is very low. So, concentration and water activity could also have an effect on the degradation of fructooligosaccharides. This study has now to be undertaken. Our degradation study was performed in buffer solutions. It is well-known that salts, including sodium, catalyze the degradation of sucrose (*23*). In this work, different buffer/salts were added to create the three different pHs. When sucrose is left to degrade at a particular pH and the pH of the reaction is monitored, there is always a decrease in pH observed because of the production of organic acid degradation products. Using buffers would have masked this effect. A study of buffered pH and non-buffered pH effects is warranted for the future. Finally, a precise study of the products that were formed throughout the degradation process is now essential to find out the mechanism whereby the reaction occurs.

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