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PRODUCTION OF OLIGOFRUCTOSE SYRUP BY ENZYMATIC HYDROLYSIS FROM INULIN-CONTAINING CHICORY SYRUP

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KEY WORDS:
oligofructose, inulin, chicory, extract, syrup, purification, enzyme preparation, hydrolysis, carbohydrate composition

ABSTRACT
The purpose of the work is to study the enzymatic hydrolysis of inulin-containing syrups of various purification degrees from chicory chips in the production of oligofructose to be used in dietary, diabetic and health-preventive nutrition products. It has been determined that ion exchange purification of the syrup is necessary for hydrolysis. Individual stages of ion-exchange purification are specified using a two-stage scheme: K1-A1-K2-A2 and an additional stage on the "Macronet" sorbent MN200 to stabilize pH and remove bitterness taste. Requirements for the quality of syrup for hydrolysis have been developed: pH value — 4.5–5.0; chromaticity — not more than 0.5 units opt. den.; ash — not more than 0.2%; protein — 0.5%; no bitterness taste. Optimal conditions for hydrolysis of inulin-containing syrup have been established using Novozim 960 endoinulinase (Denmark): temperature — 55–58 °C; pH — 4.7–5.2; DS (dry substance) — 19%; preparation dosage — 0.4 units. INU/g of syrup DS; time 20–24 h. A sample of oligofructose syrup was obtained using the preparation "Novozim 960." The carbohydrate composition of oligofructose after the inulin hydrolysis was determined: fructooligosaccharides (FOS) — 70.12%; oligofructosides — 24.79%; disaccharides — 2.11%; fructose — 2.98%. Requirements for carbohydrate composition of oligofructose obtained by enzymatic hydrolysis of inulin-containing syrup have been developed: sum total of FOS and oligosaccharides — not less than 93%, sum total of di- and monosaccharides — not more than 7%.

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1. Introduction

In the modern world one of the main directions of development of food industry is production of products for functional and health-improving nutrition based on inulin-containing types of vegetal raw materials such as chicory and girasol [1,2,3]. A promising source for the production of sugar products for functional and recreational purposes is chicory, which is characterized by a convenient form of roots, unlike tuberous girasol for processing into inulin, and also by a high inulin content.

In world practice, inulin and oligofructose as the product of its partial directed enzymatic hydrolysis, being known as widely used prebiotics for industrial use, occupy a significant place among functional food products [4,5]. Inulin and oligofructose, obtained from chicory roots, have valuable healing properties: they improve the digestive system, ensure the growth of their own intestinal bifidoflora, increase immunity, improve calcium absorption, lower blood cholesterol and even reduce the risk of bowel cancer. Inulin and oligo-fructose are also used to improve nutritional and functional properties of food products [4,5].

Thus, inulin and oligofructose are widely used as low-calorie foods of dietary and diabetic nutrition, structural formers. All this contributed to the widespread development of research on the development of inulin and oligofructose technology. In recent years, in Western Europe, their output has increased tenfold and now it amounts about 150 thousand tons per year and annually experts estimate the market growth at about 10%. Daily inulin consumption in Europe is 2–12 g/person, in the USA it is 4 g/person. [1].

Oligofructose has high solubility, but does not crystallize and does not precipitate. Therefore, it is produced both as a powder and as a syrup containing 75% dry matter. By its technological properties and taste, it is similar to sugar and can partially and completely replace it in various recipes. However, its sweetness level is only a third of the sugar sweetness, so when completely replacing sugar, oligofructose is used in combination with intense sweeteners or fructose. It is also used for diabetic nutrition, since it has an extremely low glycemic index. At the same time, it masks the aftertaste of sweeteners, improves organoleptic indicators, bringing the taste of such products to the taste of traditional products prepared using sugar as much as possible.

In the preparation of oligofructose syrups, both mineral and organic acids as well as enzyme preparations and the so-called water-soluble inulinases [6,7,8], are used as a catalyst for the partial directed hydrolysis of inulin. The advantage of acid hydrolysis is a cheaper catalyst compared to enzyme preparations. There are known studies on the use of enzyme preparations of inulinase of various origins for partial or complete hydrolysis in the production of oligofructose and fructose [9,10,11]. Studies on the use of the immobilized preparation of inulinase for the production of oligofructose are given in this work [12,13,14].

Currently, oligofructose syrups in EU countries are produced by directed partial enzymatic hydrolysis using the water-soluble endoinulinase preparation from "Novozim 960" brand Aspergillus niger ("Novozims", Denmark). According to "Orafti" (Belgium), the carbohydrate composition of "Raftilose" oligofructose in accordance with the quality certificate is as follows: the permissible mass ratio of the sum of fructooligosaccharides and oligofructosides is 93.2–97.5%, the sum of disaccharides, glucose, fructose is 2.5–6.8%. "Kosukra" (Belgium) produces the so-called "Fibrulose" food fiber with an oligofructose content of 95–99%; sum of disaccharides, glucose, fructose is not more than 5%.

According to the "Unified sanitary-epidemiological and hygienic requirements for goods subject to sanitary-epidemiological supervision (control)", the level of FOS consumption is 5–10 g/day.

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In Russia, inulin and oligofructosis are not produced. The estimated need for inulin and oligofructose only for therapeutic nutrition in the country is at least 10 thousand tons per year [2].

The All-Russian Research Institute of Starch Products is conducting scientific research on the development of a universal technology for the processing of inulin-containing raw materials for inulin and oligofructose. As a result of the studies, the most optimal sequences of technological methods for producing inulin and its derivatives — oligofructose and fructose syrup [15,16,17] were developed. The developed universal technology for processing inulin-containing raw materials into inulin consists of the following stages: purification of raw materials from impurities; washing; scalding; cutting into chips; diffusion; coagulation; mechanical filtration; purification with active carbon and ion exchange resins; cooking and drying of inulin concentrate containing no more than 85% of inulin. Powder-like inulin with higher degree of purification containing at least 95% of inulin is obtained by additional ion-exchange purification, nanofiltration or chromatography, followed by cooking and drying. Methods of purification of inulin-containing syrups using various ion-exchange adsorbents are given in studies of ARRI starch products [18,19,20]. Choice of inulin technology depends on its required purity, as well as economic prerequisites [15,21]. Partial directional enzymatic hydrolysis of inulin is required to produce oligofructose.

The purpose of the present work was to study the hydrolysis of inulin-containing syrups from chicory chips of various degrees of purification using “Novozim 960” endoinulinase enzyme preparation (Novozimes Company) for the production of oligofructose syrups.

2. Materials and methods

Inulin-containing products such as extracts, syrups of inulin oligofructose syrups from raw or dry chicory chips were used as an object of research. The extract was obtained by hot aqueous extraction from chicory chips at a temperature of 80–85 °C. After acid-thermal coagulation and filtration, the extract was purified with active coal of the OU-B brand and on “Pyrolight” ion exchange resins with strong acid cationite C150, weakly basic anionite A103S and additionally with “Macronet” sorbent MN-200. Ion-exchange purification of inulin-containing syrup from chicory was carried out according to a two-stage scheme K1+A1+K2+A2 under the following conditions: temperature — 20–35 °C; transmission speed — 1.0–2 volumes of syrup with one volume of resin per hour. In the extract and syrup purified on ion exchange resins, the pH, chromaticity, protein, ash content, carbohydrate composition, optical density in the UV spectrum (presence of such products as hydroxymethyl furfurfurol — HOMF), as well as transparency and taste (absence of bitterness) were determined. For the studies, “Raftylin” inulin of “Orafti” (Belgium) was used as a control solution for hydrolysis.

Enzymatic hydrolysis of inulin was carried out using endoinulinase preparation “Novozim 960” with activity of 400 units INU/g of the “Novozimes” company (Denmark) under following conditions: T = 55–58 °C; pH 4.7–5.2;% DS=19; dosage — 0.4 units. INU/g DS of syrup. A sample of “Raftylose” oligofructose from “Orafti” chicory (Belgium) was used to compare the results of the studies on hydrolysis of inulin-containing syrups and inulin control solution. According to the company, the carbohydrate composition of “Raftilose” oligofructose in accordance with the quality certificate is as follows: the mass fraction of the sum of fructooligosaccharides (FOS) and oligofructosides is 93.2–97.5%, di- and monosaccharides is 2.5–6.8%.

The following analysis methods and instruments were used in the studies:

- mass fraction of moisture content in inulin and oligofructose on a moisture meter MF-50 (AND company, Japan);
- mass fraction of dry solids in analysed samples of extract, syrup and solution — on a refractometer IRF-454B2M;
- determination of the carbohydrate composition of extract, syrup, inulin and oligofructose on a liquid chromatograph of carbohydrates with a Gilson refractometric sensor;
- determination of the optical density of syrups — on a spectrophotometer SF 2000 (Design Bureau “Spectrum”, St. Petersburg);
- chrominance determination — on photoelectrocorimeter KFK-3;
- weight fraction of protein by Kjeldahl method on a K-424 apparatus (BUCHI Labortecknik, Germany);
- determination of ash weight fraction, pH value and other parameters — according to methods adopted in the starch and beet sugar production [22].

3. Results and discussion

The article presents the results of studies on enzymatic hydrolysis of inulin-containing syrups from chicory chips of various degrees of purification to produce oligofructose syrups. Hydrolysis of the solution of inulin and inulin-containing syrups after ion-exchange purification, as well as chicory chips extract, was carried out using the “Novozim 960” enzymatic preparation endoinulinase from Aspergillus niger (Denmark). Enzyme activity is 400 units. INU/g preparation. Hydrolysis results were evaluated by the carbohydrate composition of the products obtained.

3.1. Development of requirements for the purification degree of chicory chips syrup for hydrolysis

In order to study the effect of the degree of purification of the initial inulin-containing syrup on hydrolysis in the production of oligofructose, studies were carried out to elaborate separate steps of ion-exchange purification of chicory extracts and syrups using “Purelight” resins (USA). The original extract obtained by aqueous diffusion and purified by acid-thermal coagulation was characterized by a high content of impurities and chromaticity: the weight fraction of protein is 4.2%; ash — 5.1%, chromaticity — 3.50 comp. units opt. den. Due to the presence of intibin glycoside in it, he had a strong taste of bitterness.

It was established that the bitterness taste of chicory extract during its purification using the “Macronet” sorbent MN200 completely disappears [18]. In this regard, to study the effect of the degree of purification of the initial extract on hydrolysis, it was purified without double ion exchange purification using only the “Macronet” sorbent MN200. The results of studies on the purification of chicory extract on the “Macronet” adsorbent showed that its chromaticity after purification decreased by more than 50%, as evidenced by a decrease of optical density in the ultraviolet region of the spectrum at a wavelength of 275–280 nm. The results of the UV spectrum extract analysis before and after purification are as follows (Figure 1) on the “Macronet” sorbent.

From Figure 1, it follows that the content of compounds like hydrooxymethylfurfurfurol-HOMF type (optical density γ = 275–285 nm), giving high chromaticity to the extract, after purification on the “Macronet” adsorbent MN200, decreased by 2.2 times. The absence of a peak in the purified product at this wavelength indicates a significant decrease of colorants content.

In addition, it should be noted that the mass fraction of protein, ash elements and other impurities in the extract from chicory purified on the “Macronet” adsorbent MN200 remained at a high level, but this stabilizes the pH (4.7–5.0). Therefore, further, in preparing the syrup for hydrolysis, it is not necessary to further adjust the pH to the optimal value for the action of the used enzyme preparation inulinase.

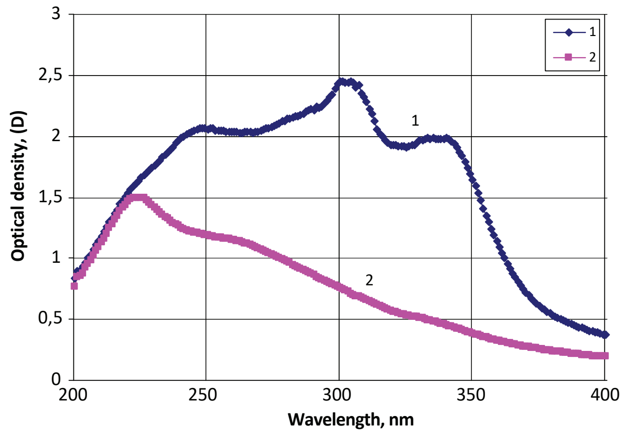

Figure 1. Optical density (D) versus wavelength: 1 — initial chicory extract; 2 — chicory extract after purification on the "Macronet" adsorbent MN200

To improve the quality of chicory extract purification and to remove the above impurities, preliminary double ion-exchange purification of the extract according to the K1-A1-K2-A2 scheme was carried out using resins: strong acid cationite grade C150, weakly basic anionite grade A103S. Despite satisfactory results in the removal of impurities ash content in the product is reduced to 0.2%, and the weight fraction of protein is up to 0.5%, the discoloration effect is 87%, however, there was a bitterness taste in the syrup. To remove the bitterness taste of the syrup, it was additionally cleaned on the "Macronet" adsorbent MN200.

Thus, it has been found that in preparation of chicory syrup for the hydrolysis, with the enzymatic preparation of inulinase it is required obligatory double ion exchange purification. At the same time, it is possible to reduce the mass fraction of impurities such as protein, colorants and minerals by ten times. At the final stage of syrup purification it is necessary to use "Macronet" sorbent MN200 for its additional purification, removal of bitterness taste and stabilization of pH syrup before hydrolysis.

As a result of studies, requirements for the quality of chicory syrup after the above purification methods for obtaining oligofructose by enzymatic hydrolysis using inulinase brand "Novozim 960" were developed:

- pH value — 4.5–5.0;
- chromaticity — not more than 0.5 units opt. square;
- ash mass fraction — not more than 0.2%;
- protein weight fraction — 0.5%;
- lack of taste of bitterness.

In the preparation of oligofructose syrup, "Raftylin" inulin of "Orafti" (Belgium) was used as a control solution for the hydrolysis.

As a result of studies for enzymatic hydrolysis in the production of oligofructose syrup, quality indexes of both the control solution of inulin "Raftylin GR" and the inulin-containing products from chicory chips of various purification degrees were determined (Table 1).

The studies showed that the maximum degree of purification and the optimal carbohydrate composition (minimum amount of disaccharides and absence of monosaccharides) has a control solution of inulin "Raftylin GR" (Table 1). After the two-stage ion-exchange purification of extract, weight fraction of impurities in the purified syrup decreases tenfold. However, after ion exchange purification, fructose appears in the syrup, which is undesirable in the production of oligofructose. The extract from chicory chips after purification on the "Macronet" sorbent MN200 has a high content of impurities (ash and protein). Attention should be paid to the carbohydrate composition of the obtained samples. The total weight fraction of di- and monosaccharides in the initial syrups is significantly different from the control sample. In the chicory syrup after ion-exchange purification and, especially in the chicory chips extract, their weight fraction is more than 12%, and in control inulin solution only 5.76%.

Table 1

Characteristics of inulin-containing products of varying degrees of purification for hydrolysis in the production of oligofructose

Sample name	Mass fraction, %		Weight fraction of carbohydrates, %				
	ash	protein	inulin	oligo-fructosides	disaccharides	glucose	fructose
Control solution of inulin «Raftylin»	0.03	0.00	89.25	4.99	5.76	0.00	0.00
Chicory extract after cleaning on "Macronet"	3.1	4.5	78.38	9.20	9.72	0.00	2.7
Chicory syrup after purification K_1-A_1-K_2-A_2	0.2	0.5	83.24	4.51	6.85	0.00	5.39

3.2. Hydrolysis studies on chicory chips syrup of various purification degrees

Hydrolysis of the control solution of inulin, the inulin-containing extract and the syrup after the above purification methods was carried out with the enzymatic preparation of endoinulinase "Novozim 960." Hydrolysis of inulin to produce oligofructose was carried out under the following conditions: T = 55–58 °C; pH — 4.7–5.2; DS = 19–20%; dosage of the preparation 0.4 units. INU per DS syrup; time — 24 h. To compare the obtained results after the hydrolysis of inulin, a chicory oligofructose was used as a control sample.

After hydrolysis of the above samples of inulin-containing syrups with various degrees of purification, the carbohydrate composition of oligofructose syrups and the "Raftylose" sample was determined (Table 2).

Table 2

Carbohydrate composition of samples of "Raftylose" oligofructose and oligofructose syrups after hydrolysis

Sample name	Carbohydrate composition of samples after hydrolysis,%						
	FOS	oligofructosides	ΣFOS and oligofructosides	disaccharides	glucose	fructose	Σ di- and monosaccharides
"Raftiloza"	70.12	24.79	94.91	2.11	0.00	2.98	5.09
Syrup from «Raftylin» inulin	73.04	22.79	95.83	1.74	0.00	2.43	4.17
Chicory syrup after «Macronet»	62.23	25.69	87.92	2.87	4.01	5.20	12.08
Chicory syrup after K_1-A_1-K_2-A_2	60.82	31.93	92.75	1.96	2.27	3.02	7.25

Comparison of the carbohydrate composition after hydrolysis of chicory syrup of various purification degrees (on the "Macronet" adsorbent and after K1-A1-K2-A2) with the carbohydrate composition of the control sample of "Raftilose" oligofructose shows a significant effect of impurities of the initial syrup and its carbohydrate composition on enzymatic hydrolysis. The optimal carbohydrate composition (a small amount of disaccharides and the absence of monosaccharides) in the "Raftylin GR" solution of inulin makes it possible to obtain an optimal composition for oligofructose, similar to the "Raftylose" oligofructose.

When using an extract purified only on the "Macronet" adsorbent (Table 2), hydrolysis proceeds to form a small amount of oligofructosides and an increased fructose and glucose content. The sum total of FOS (fructooligosaccharides) and oligofructosides is below 90% (87.92%), and the sum total of di- and monosaccharides has a high value — 12.08%. This is probably due to the presence in the original extract for hydrolysis of a high content of oligofructosides, disaccharides — a substrate for hydrolysis, as well as fructose. The increased weight fraction of impurities in the extracts (protein — 6.8%, ash — 3.2%), as well as its carbohydrate composition do not contribute to the optimally required carbohydrate ratio during hydrolysis of the inulin-containing extract.

Thus, in the hydrolysis of an extract from chicory with a low degree of purification (only using the "Macronet" sorbent), it is not possible to achieve the required quality of the carbohydrate composition of the oligofructose syrup, both due to the influence of the contained impurities and its carbohydrate composition.

It was found that after a two-stage ion exchange purification (Table 1) content of mono and disaccharides in chicory syrup is higher compared to the control sample of inulin and makes: disaccharides — 6.85% and fructose — 5.39%. Despite this, hydrolysis of chicory syrup after ion exchange purification (Table 2) proceeds with the formation of an optimal carbohydrate composition: the sum total of FOS and oligofructosides is 92.75%, and the sum total of di- and monosaccharides is 7.25% (including disaccharides — 1.68%, glucose — 2.27%, fructose — 3.02%).

The obtained results indicate the need to control the carbohydrate composition of the initial syrup for hydrolysis (absence of glucose, minimum content of mono- and disaccharides, as well as thorough purification of inulin-containing syrup from impurities before hydrolysis).

To obtain oligofructose syrup with optimal ratio of carbohydrate composition for di- and monosaccharides, it is necessary to use initial inulin-containing syrup containing no glucose for hydrolysis, disaccharides — less than 5.5%, as well as the sum total of mono- and disaccharides — less than 7%.

Thus, during enzymatic hydrolysis of chicory syrup, a thorough ion exchange purification according to a two-stage scheme is required to obtain oligofructose: K1-A1-K2-A2 and additionally on the "Macronet" sorbent MN200 to stabilize the pH value and remove bitterness flavor, as well as control over the carbohydrate composition of the initial syrup for hydrolysis.

In laboratory conditions, a sample of oligofructose syrup was obtained using the above scheme for purifying chicory chips syrup before hydrolysis. The following is a carbohydrate composition of the laboratory sample (Table 3) obtained at a dosage of 0.4 INU/g DS, a hydrolysis duration of 20 hours and a control sample of "Raftilose" oligofructose. The laboratory sample of oligofructose syrup differs from the control one by a slight increase in the sum total of di- and monosaccharides — 6.82%, the sum total of FOS and oligofructosides corresponds to the required value — 93%.

Table 3

Carbohydrate composition of oligofructose samples

Sample Name	Carbohydrate composition, %						
	FOS	oligofructosides	∑ FOS and oligofructosides	disaccharides	glucose	fructose	∑ di- and monosaccharides
Control sample "Raftylose"	70,12	24,79	95,01	2,11	—	2,98	5,09
Laboratory sample	70,85	22,32	93,17	3,24	—	3,58	6,82

The obtained data correspond to the quality certificate indexes for "Raftylose" oligofructose of "Orafti" Company for the mass fraction of the sum total of FOS and oligofructosides, as well as di- and monosaccharides. That indicates the absence of glucose in both control and laboratory samples.

Based on the studies, the requirements for the carbohydrate composition of oligofructose from inulin-containing syrup were developed: the sum total of high-molecular fructooligosaccharides (FOS) and oligofructosides is at least 93%, the sum of low-molecular saccharides (di- and monosaccharides) is not more than 7%.

The significance of the work is the creation of domestic technology of oligofructose from inulin-containing chicory extract as a component for therapeutic and preventive nutrition of the population and ensuring its import substitution

4. Conclusion

To study the effect of the degree of purification of the initial inulin-containing syrup on hydrolysis in the production of oligofructose, studies were carried out to clarify individual steps of ion-exchange purification of chicory extracts and syrups using "Purelight" resins (USA).

Separate steps of ion-exchange purification of chicory extracts and syrups in production of oligofructose syrup are specified. It was established that in preparation the syrup for enzymatic hydrolysis it is required an obligatory double ion exchange purification and an additional purification are on the "Macronet" sorbent MN200 to remove bitterness flavor and stabilize the pH of the syrup. At the same time, it is possible to reduce the mass fraction of such impurities as protein, colorants and minerals by ten times.

Requirements for the quality of syrup after ion-exchange purification for hydrolysis with the enzyme preparation inulinase have been developed: pH — 4.5–5.0; chromaticity — not more than — 0.5 units opt. square; ash mass fraction — not more than 0.2%; protein weight fraction — 0.5%; lack of bitterness.

Enzymatic hydrolysis of inulin-containing syrup was carried out under the following conditions: T = 55–58 °C; pH — 4.7–5.2; % DS=19; dosage — 0.4 units. INU/g DS syrup, duration 20–24 h. A sample of "Raftylose" oligofructose from "Orafti" chicory (Belgium) was used to compare the hydrolysis results of the control solution of "Raftilin" inulin and inulin-containing syrups.

Comparison of obtained results on hydrolysis of inulin solution of "Raftylin" brand, extract after purification only on "Macronet" sorbent MN200 and syrup after double ion-exchange purification shows significant influence of impurities of initial syrup and its carbohydrate composition on enzymatic hydrolysis.

With the hydrolysis of "Raftylin GR" inulin, its maximum degree of purification and optimal carbohydrate composition allow to obtain the carbohydrate composition required for oligofructose, similar to "Raftylose" oligofructose.

The hydrolysis of chicory syrup after ion exchange purification also proceeds with the formation of an optimal carbohydrate composition: the sum total of FOS and oligofructosides is 92.7%, and the sum total of di- and monosaccharides is 7.25%.

The obtained results indicate the need to control the carbohydrate composition of the initial inulin-containing syrup before hydrolysis (absence of glucose, content of di- and monosaccharides — less than 7%).

Thus, during the enzymatic hydrolysis of chicory syrup, a thorough ion exchange purification according to a two-stage scheme is required to obtain oligofructose: K1-A1-K2-A2 and additionally on the "Macronet" sorbent MN200 to stabilize the pH and remove bitterness flavor.

A laboratory sample of oligofructose from chicory syrup purified according to the proposed scheme is obtained, the carbohydrate composition of which corresponds to the data of the quality certificate for the sample of oligofructose brand "Raftilose" according to the weight fraction of the sum total of FOS and oligofructosides, as well as di- and monosaccharides.

Based on the studies, the requirements for the carbohydrate composition of oligofructose from inulin-containing syrup were developed: the sum total of FOS and oligofructosides — at least 93%, the sum total of di- and monosaccharides — no more than 7%.

In Russia the organization of the production of inulin, oligofructose and food products based on them is an important national economic task. Due to the lack of production of inulin and oligofructose from inulin-containing raw materials in Russia, the development of their technology is very relevant and promising.

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