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Original research paper

ENZYMATIC SYNTHESIS OF FRUCTO-OLIGOSACCHARIDES USING PECTINEX® ULTRA SP-L: A STUDY OF EXPERIMENTAL CONDITIONS

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Abstract: The obvious benefits of employing prebiotics as functional components in many foods and feed products have resulted in higher demand for their industrial production, necessitating the development of more efficient and cost-effective manufacturing procedures. As a result, the goal of this study was to synthesize confirmed prebiotics, namely fructo-oligosaccharides (FOS), using sucrose as a substrate, since it allows the synthesis of oligosaccharides with lower polymerization degree, and consequently, a more pronounced prebiotic effect. Due to its availability, low market price, and high stability under industrial conditions, a commercial enzymatic mixture, Pectinex® Ultra SP-L, is used as a source of enzyme – fructosyltransferase (FTase). By varying key experimental conditions such as pH, temperature, enzyme and substrate concentrations, as well as the duration of the process, the composition of the FOS mixture can be adjusted to fit the potential applications. It was found that by performing the reaction in an aqueous medium (pH 7), at a temperature of 50 °C using an enzyme concentration of 1% (v/v) and any sucrose concentration in the range of 200-700 g/L, it was possible to achieve maximum FOS yield of 60% of total carbohydrates within a 24 h. The produced syrup with a high content of FOS can be further used as an adequate food additive, or else, developed processes should be used for the transformation of various food products (such as juices, jams, fillings, candies, cakes, etc.) in which sucrose dominates, creating products with lower caloric and higher functional value.

Key words: *fructo-oligosaccharides, prebiotics, fructosyltransferase, enzymatic synthesis*

INTRODUCTION

According to the most recent scientific definetion, dating from 2016, a prebiotic is defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al., 2017). Therefore, this versatile group of compounds has received much attention in recent years due to their importance, not only for the gastrointestinal tract (GIT), but also for the whole organism (Davani-Davari et al., 2019). Because they are non-digestible, they reach the large intestine unchanged where they represent a source of food for a certain group of beneficial microorganisms (Ashwini et al., 2019), providing their proliferation and ensuring the normal functioning of the human

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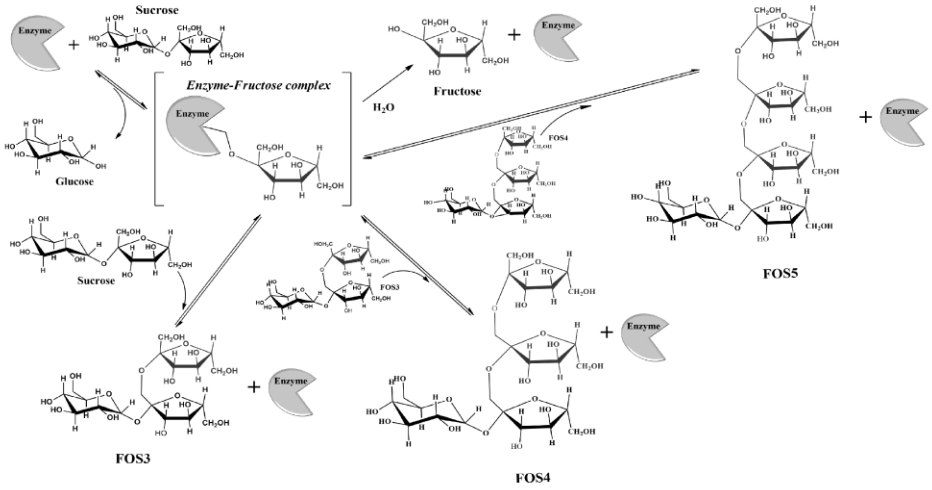
gut (Hernandez & Pandiella, 2013). Additionally, compounds that prevent pathogenic organisms from surviving, have a favorable influence on the immune system, mineral absorption, and may accomplish other health benefits are produced as a result of their fermentation (Ashwini et al., 2019; Campos-Perez & Martinez-Lopez, 2021).

Fructo-oligosaccharides (FOS) are considered to be confirmed prebiotics and present the group of oligosaccharides that are naturally present in various fruit and vegetables (Maiorano, Piccoli, Da Silva & De Andrade Rodrigues, 2008; Sridevi, Sumathi, Guro Prasad & Murari, 2014). The FOS is made up of one terminal glucose and several other fructose units. Due to their nutritional properties (non-cariogenic, non-caloric, low intensity of sweetness compared to sucrose) and numerous confirmed positive effects on human health, they are considered quite useful for application in products of the food and feed industry (Tungland, 2003; Kumar & Dubey, 2019; Martins, Ureta, Tymczyszyn, Castilho & Gomez-Zavaglia, 2019). It has been demonstrated that FOS can contribute to humectant in baked products, enable decrement of the freezing point in frozen food products, provide crispness to cookies, and act as a binder in nutritional or granola bars, and they are particularly interesting for products in which the use of sucrose is undesirable, such as diabetic products (Kaur & Gupta, 2002). Also, new food regulations that recommend the elimination of different chemical additives in food preparations raise interest in the use of FOS as natural preservatives and low-calorie sweeteners, based on their cost-effectiveness and superior properties (Romano, Santos, Mobili, Vega & Gómez-Zavaglia, 2016). Likewise, they are becoming an unavoidable supplement in animal feed and pet food preparations, where they ensure better digestion and a healthier gut, and consequently act as growth promoters by enhancing feed efficiency (Shang, Kumar, Thippareddi & Kim, 2018).

Having said that, the development of prebiotic products, which have been in the spotlight for the past decade, has resulted in a dramatic increase in demand and growth of production. Due to the low content of FOS in natural sources, they are obtained through chemical or enzymatic means, primarily through inulin hydrolysis or sucrose transformation (Dominguez, Rodrigues, Lima & Teixeira, 2014). The use of enzymes is often preferred due to their high specificity and the fact that this process generates less waste and by-products (Sánchez-Martínez, Soto-Jover, Antolinos, Martínez-Hernández & López-Gómez, 2020). Depending on the starting substrate and method of production, the oligosaccharides of different structures and chain lengths can be obtained (Sridevi et al., 2014). The synthesis of FOS from sucrose is frequently pursued, not only for the benefit of obtaining oligosaccharides with lower molar masses, which have been scientifically proven to have a more pronounced prebiotic effect (Martins et al., 2019), but also for economic reasons, as sucrose is regarded as an abundant and inexpensive substrate (Sánchez-Martnez et al., 2020). Enzymatic synthesis of FOS from sucrose can be achieved using enzymes with transfructosylating activity such as fructosyltransferase (FTase, EC 2.4.1.9) or *β*-fructofuranosidase (FFase, EC 3.2.1.26) under specific conditions (Smaali et al., 2012). Owing to the fewer prerequisites and high productivity, FTases proved to be the enzymes of choice (Kashyap, Palai & Bhattacharya, 2015). Additionally, they are widespread (Singh, Jadaun, Narnoliya & Pandey, 2017), and often appear along with other enzymes like pectinases and cellulases in their commercial preparations (e.g. Pectinex® Ultra SP-L and Viscosyme® L).

Even though FOS have been extensively studied, the mechanism of FOS synthe-sis *via* FTase catalysed reaction is still not completely resolved (Scheme 1) (Flores-Maltos et al., 2016; Kumar & Dubey, 2019). It is proposed that FOS synthesis starts with the cleavage of the sucrose glycosidic bond and the transfer of the fructosyl unit to a wide range of different acceptors. More precisely, another sucrose molecule or previously synthesized FOS might serve as an acceptor of the fructosyl moiety, resulting in the generation of a complex FOS mixture (Contesini et al., 2018). Simultaneously, but to a significantly lesser extent, water molecules act as acceptors, leading to hydrolysis reactions (Martins et al., 2019). Upon completion of the reaction, the reaction mixture contains FOS of different degrees of polymerization in the largest amount, then glucose which is released as the by-product, as well as insignificant amounts of sucrose and fructose. It is generally known that the transfructosylation reaction is kinetically controlled



Scheme 1. Enzymatic synthesis of FOS from sucrose

and that the composition of the resulting mixture can be modulated by varying the process conditions (Vega & Zúniga-Hansen, 2011). Hence, the main goal of this study was to examine the influence of key reaction conditions on FOS synthesis, using the commercial preparation Pectinex® Ultra SP-L (with FTase activity) and develop a cost-effective process that can be further transferred to various other substrates rich in sucrose to produce *in-situ* derived prebiotic-containing food preparations.

MATERIALS AND METHODS

Materials

For the production of FOS, as a substrate, sucrose (Thermo Fisher Scientific, Waltham, USA) was used, while commercial enzymatic mixture Pectinex® Ultra SP-L (Novozymes, Bagsvaerd, Denmark) was used as a source of FTase. Substances used for buffer preparation (sodium acetate, sodium hydrogen phosphate, sodium dihydrogen phosphate, acetic acid, hydro-chloric acid and sodium hydroxide) were purchased from Centrohem (Stara Pazova, Serbia). Water and acetonitrile (HPLC grade) used for HPLC analyses, were purchased from Thermo Fisher Scientific (Waltham, USA).

Enzyme activity assay

FTase activity from the enzymatic mixture Pectinex® Ultra SP-L, was determined by performing reaction of FOS synthesis in Erlenmeyer flask volume of 50 ml on an orbital shaker (IKA® KS 4000i control, Werke GmbH and Co., Staufen, Germany) at 120 rpm. From the reaction medium (5 ml) composed of sucrose solution (500 g/L) and enzyme preparation (100 µl), samples were taken within the first 30 min to ensure the initial kinetics, diluted with distilled water and treated for 5 min in ThermoMixer® C (Eppendorf, Hamburg, Germany) at 100 ºC to inactivate the enzyme. The samples were then filtered and analyzed by HPLC system. One unit of FTase activity (IU) was defined as the amount of enzyme which catalyzes the formation of 1 µmol FOS per min under defined reaction conditions.

Enzymatic synthesis of FOS

All reactions of FOS synthesis were performed in Erlenmeyer flasks on an orbital shaker at 120 rpm. The reacton mixture consisted of 50 ml of the substrate (sucrose solution) and a certain amount of the biocatalyst. To enhance the process of enzymatic synthesis, reactions were performed in mediums with different pH (4.0-8.0) at various temperatures (30-70 °C) and using different concentrations of enzyme (1, 2 and 5% v/v) and substrate (200-700 g/L). It is important to emphasize that during the execution of the each experiment in which the individual parameters were varied, the others were kept constant in order to determine the best suited conditions for FOS synthesis.

At predefined reaction time, the samples were taken, diluted with distilled water and treated for 5 min in ThermoMixer® C at 100 ºC to inactivate the enzyme. After that, the samples were filtered and analyzed by HPLC system.

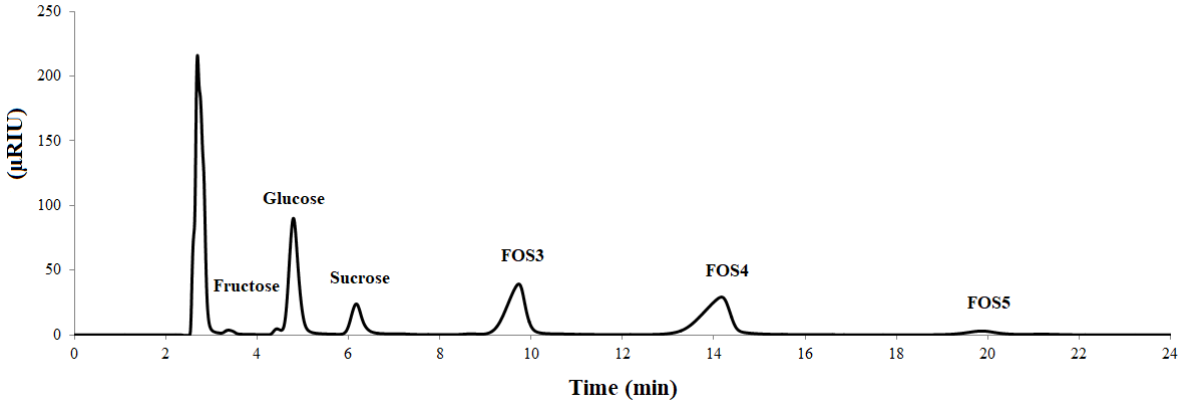


Figure 1. Characteristic chromatogram of the reaction mixture: fructose (t_r = ~ 4.4 min), glucose (t_r = ~ 4.8 min), sucrose (t_r = ~ 6.2 min), trisaccharides- FOS3 (t_r = ~ 9.7 min), tetrasaccharides FOS4 (t_r = ~ 14.2 min) and pentasaccharides FOS5 (t_r = ~ 20.0 min), where t_r present retention time of individual components

Quantitative analysis

Analysis of the composition of the reaction mixture was performed by Dionex Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, USA). For this purpose, an amino column (Hypersil™ APS-2, 250 mm × 4 mm, 5 μm) at 35 °C was used. An 80% (v/v) aqueous solution of acetonitrile with a flow rate of 1 ml/min was used as a mobile phase. Product detection was performed using an RI detector (RefractoMax 520). Data collection and further processing were performed using Chromeleon 7.2 Data System. Glucose, fructose, sucrose, 1-kestose and nystose were utilized as standards for determination of reaction mixture components. A characteristic chromatogram of the obtained reaction mixture, with defined retention times for each reaction mixture component, is shown in Fig. 1.

RESULTS AND DISCUSSION

At the beginning of the experimental work, the complexity of FOS synthesis (Scheme 1), which involves a series of consecutive and parallel reactions, was examined by monitoring the profiles of all participants in the reaction. Primarily, it can be observed that the concentration of sucrose, which is spent on the synthesis of FOS, steadily decreases during the whole examined period (Fig. 2a). This decrement becomes less pronounced after reaching approximately 100 g/L (around 10h). At the same time, the concentration of total FOS rises sharply in the first 10 h, while afterwards enters the stationary phase. However, this stagnation period of the total FOS concentration could be better understood by a detailed overview of the concentration profiles of FOS of different polymerization degrees (Fig. 2b). It could be noted that the generation of FOS3 (kestose) occurs first and that for the first 6 hours, this component is the major reaction product. After reaching a certain concentration, FOS3 gains the role of a fructosyl moiety acceptor, and its steep growth is slowed down upon its consumption for FOS4 (nystose) formation, whereas the synthesis of FOS3 is still ongoing. At some point, around 12 h, the FOS3 consumption prevailed, and in this period FOS4 synthesis becomes prominent. The synthesis of FOS5 starts at the end of the examined reaction period, after reaching ap-propriate concentrations of its substrate (FOS4). Moreover, the reaction mixture contains a certain amount of glucose and fructose, as well as unreacted sucrose, that represent undesirable by-products of the reaction. However, as depicted in Fig. 2a, fructose concentration remains negligible throughout the whole examined period, indicating the dominance of transfructosylation over the reaction of hydrolysis.

Similar results were observed previously for this type of enzyme (FTase), thus, proving its adequacy for FOS synthesis (Kashyap et al., 2015).

Given the above-mentioned complexity of FOS synthesis, it is clear that careful study of the most important reaction conditions is required to highlight the transfructosylation reaction and achieve the desired reaction mixture composition. As a result, the following set of

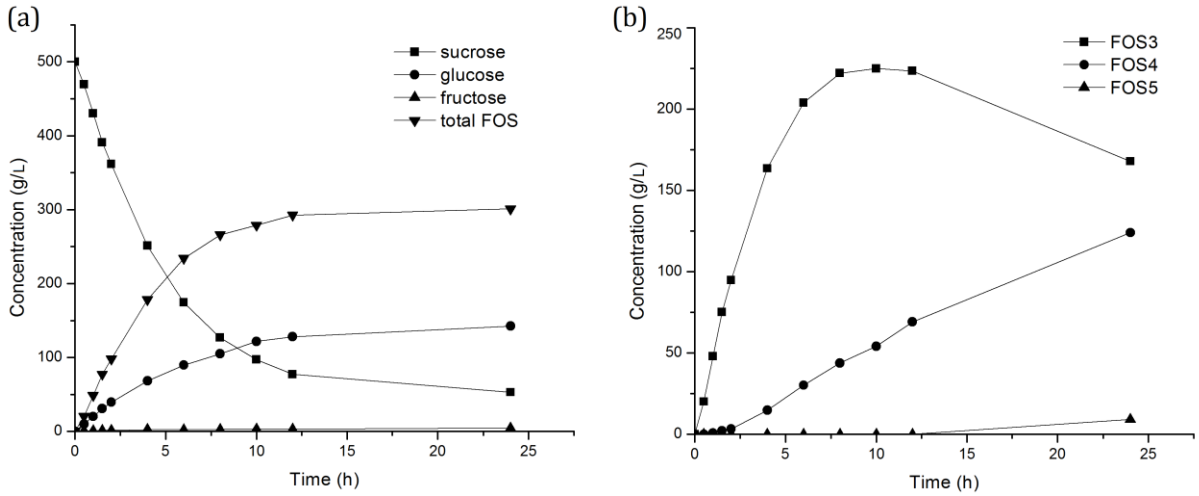


Figure 2. Time course of FOS production: (a) FOS concentration and (b) FOS yield. Experiments were performed using sucrose solution (500 g/L) prepared with 0.1M sodium acetate buffer (pH 5.5) with 1 % (v/v) of enzymatic preparation Pectinex® Ultra SP-L at 50 °C

experiments will focus on studying FOS enzymatic synthesis by varying key reaction factors like temperature, pH, enzyme, and substrate concentrations individually.

Determination of the pH and temperature effects on FOS synthesis

It is known that the effect of experimental conditions such as pH and temperature is highly dependent on the nature of the used enzyme (Martins et al., 2019). According to available research, the temperature is a variable that can have a significant impact on the rate of an enzymatic reaction, while having a minor impact on the overall concentration of the product (Vega & Zniga-Hansen, 2011). The reaction rates increase, as the temperature rises (Bisswanger, 2014), yet performing the reaction at very high temperatures might produce thermal damage to the enzyme, resulting in a large drop in activity (Bisswanger, 2014; Martins et al., 2019).

FOS synthesis with FTase is possible across a wide range of temperatures (Martins et al., 2019), while generally higher temperatures are preferred due to the increased sucrose solubility, prevented microbial contamination, lowered viscosity and improved reaction rates (Nemukula, Mutanda, Wilhelmi & Whiteley, 2009). To determine the best working temperature using FTase from Pectinex® Ultra SP-L, the reaction was performed in the temperature range of 30-70 °C. The obtained results suggest that with the increasing temperature, the initial reaction rate increases significantly, directly affecting the time of reaching the maximum FOS concentration (Fig. 3). This trend is also in line with the results of the analysis reported by Vega and Zúniga-Hansen (2011). Although slight differences in FOS concentrations (approximately 300 g/L, corresponding to yields of approximately 60% of FOS in total carbohydrates) were achieved at the mentioned temperatures, higher temperatures of 60 °C and 70 °C yielded maximum FOS concentrations after 8 h, while the same concentration was reached after 24 h at 30 °C. It is interesting to note that FTase from Pectinex® Ultra SP-L in another study showed exceptional thermal resistance by performing reactions at 70 °C and achieving FOS yields of 56%, although some authors state that in general, enzymes like FTases can be thermally damaged by performing reactions at temperatures above 60 °C (Martins et al., 2019). Given that performed experiments represent preliminary results based on reaction using pure sucrose solutions, and because of future utilization in the transformation of more complexed substrates that might be prone to colour changing upon treatments at high temperatures, the temperature of 50 °C, where highest yields are achieved in 12 h, was chosen as the most suitable. The effect of pH on the enzymatic production of FOS was investigated by varying pH values between 4.0 and 8.0. Figure 4 illustrates the acquired results. These findings are consistent with those reported by Vega and Zniga-Hansen (2011), and they support the conclusion that pH has only a

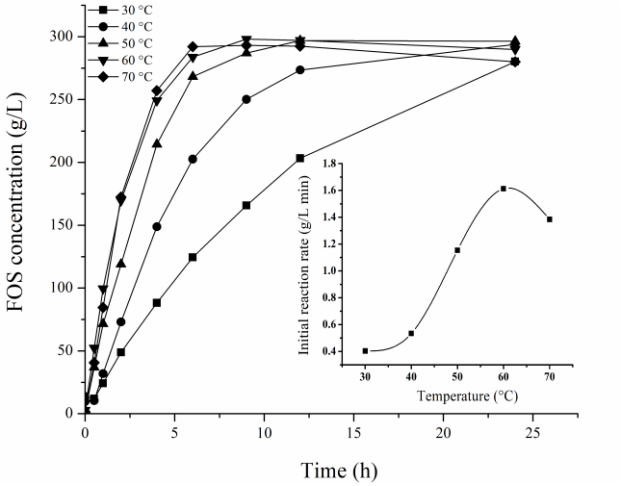


Figure 3. Effect of temperature on FOS production. Experiments were carried out in sucrose solution (500 g/L) prepared with 0.1M sodium acetate buffer (pH 5.5) with 1% (v/v) at different temperatures (30-70 °C). Insert graph illustrates the dependence of the initial reaction rate on the temperature of reaction

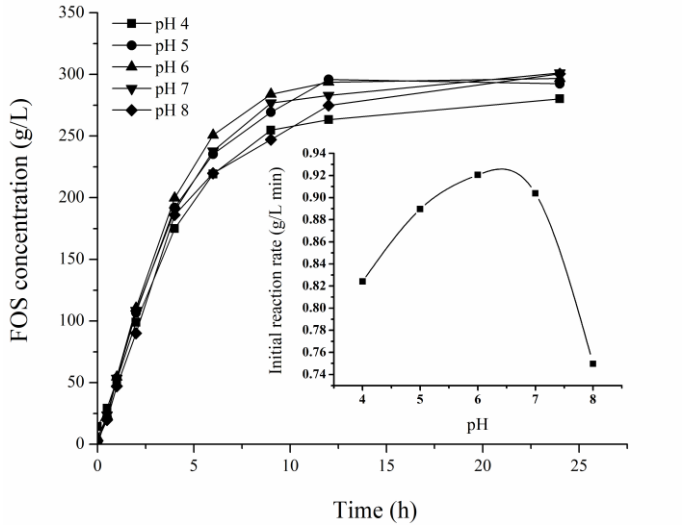


Figure 4. Effect of pH on FOS production. Experiments were carried out in sucrose solution (500 g/L) prepared with 0.1M sodium acetate buffer (pH 4.0-5.0) and 0.1 M sodium phosphate buffer (pH 6.0-8.0), with 1% (v/v) of enzyme at 50 °C. Inset graph illustrate the dependence of the initial reaction rates on the different pH

minor impact on product output and initial reaction rates. As a result, it can be concluded that this enzyme preparation is particularly suited for the formation of FOS at all pH values higher than 4 because the obtained concentrations increase with Namely, the enzymes are very sensitive to changes in pH, because the change in pH can lead to a change in the state of ionization of amino acids that are an integral part of the enzyme structure.

Accordingly, the shape and structure of the protein can be altered, which consequently leads to a change in activity or complete inactivation of the enzyme (Battestin & Macedo, 2007). Therefore, changes in the pH of the medium affect the activity of the enzyme (Bisswanger, 2014), primarily by changing the enzymatic reaction rate, while the influence of this factor on the product yield is often insignificant (Vega & Zúniga-Hansen, 2011). increasing reaction time, and the highest produced GOS concentrations were approximately 300 g/L. Previous reports state that hydrolytic activity of the enzyme may prevail at a pH around 4, causing lower FOS yields (Nemukula et al., 2009). Nonetheless, it is worth noting that this enzyme preparation is primarily intended for purposes of pectin degradation in fruits and vegetables under acidic conditions (Rodrigues, Carvalho, & Rocha, 2014), and in light of future applications that

Table 1.
Effect of the enzyme concentration on: a) FOS production and b) productivity. Experiments were carried out in sucrose solution (500 g/L) prepared with distlled water at 50 °C

Time (h)	**1% (v/v)**		**2% (v/v)**		**5% (v/v)**	
	FOS concentration (g/L)	**Productivity** (g FOS/ml enzyme)	**FOS concentration** (g/L)	**Productivity** (g FOS/ml enzyme)	**FOS concentration** (g/L)	**Productivity** (g FOS/ml enzyme)
0	1.917	0.192	6.237	0.312	4.976	0.100
0.5	21.540	2.154	39.088	1.954	81.018	1.620
1	55.481	5.548	86.650	4.332	163.816	3.276
2	107.603	10.760	162.802	8.140	254.763	5.095
4	181.412	18.141	250.310	12.515	298.659	5.973
6	231.332	23.133	292.146	14.607	297.575	5.951
9	284.134	28.413	305.198	15.260	289.695	5.794
12	295.253	29.525	297.626	14.881	288.392	5.768
24	302.178	30.218	289.736	14.487	262.534	5.251

may require the use of this enzyme preparation in such complex preparations, the neutral pH values, namely pH 7, could represent the best choice preparations, the neutral pH values, namely pH 7, could represent the best choice. In this way, the undesired pectinolytic activity could be suppressed to some extent, allowing usage of enzyme preparation without the need for performing tedious prior purification steps. Additionally, the chosen pH value permits carrying out reactions in a water environment, making the process more user-friendly and more acceptable for the food industry.

Determination of the enzyme concentration effect on FOS synthesis

Many studies state that enzyme concentration is also a factor that does not greatly affect the yield of the products, but affect the velocity of the enzymatic reaction (Hang & Woodams, 1996; Vega & Zúniga-Hansen, 2011; Kashyap et al., 2015). Examining the influence of this factor on the enzyme synthesis of FOS by varying the concentrations (1, 2, and 5 % v/v), we obtained results that confirm the observation of Vega and Zúniga-Hansen (2011) and Kashyap et al. (2015) that the use of higher enzyme concentration achieves a higher initial reaction rate and, therefore, a shorter time is expected to reach the maximum yield. From Table 1, it can be seen that the highest values of FOS concentration, in the case of the use of enzymes of 1% (302 g/L), 2% (305 g/L) and 5% (298 g/L) were achieved after 24, 9 and 4h, respectively. The explanation of the obtained results lies in the fact that with increasing enzyme concentration, the number of active sites available to the substrate increases, which increases the reaction rate, and a decrease in the time needed to achieve the maximum yield (Creative Enzymes, 2021).

To more reliably determine the impact of these reaction conditions, a dependence of reaction time and enzyme concentration on productivity is presented (Table 1). Opposite trend can be noticed, thus, with increasing enzyme concentration productivity decreases. Taking into account the obtained results and more importantly by calculating specific reaction rates (g FOS/ml enzyme/h) achieved in these experiments: 2.46 (1%), 1.69 (2%), and 1.49 (5%), a concentration of 1% (v/v) was chosen as best suited because the use of smaller amounts of enzymes achieves appropriate effects and reduces the cost of the synthesis process.

Determination of the substrate concentration effect on FOS synthesis

In the final stage of research, bearing in mind that substrate concentration has proved to be a key experimental factor whose variation can significantly affect FOS production (Vega & Zúniga-Hansen, 2011), the influence of sucrose concentration in the range of 200-700 g/L was examined. The progress curves were generated by analyzing the effect of substrate concentration on the enzymatic production of FOS through two different outputs, FOS concentration (g/L) and FOS yield (%), as illustrated in Fig. 5. It can be seen that the use of a higher substrate concentration directly influences the formation of a higher amounts of FOS. For the examined substrate concentration range, maximum achieved yields vary from 116 g/L to 415 g/L. These results are consistent with the findings of previously pu-

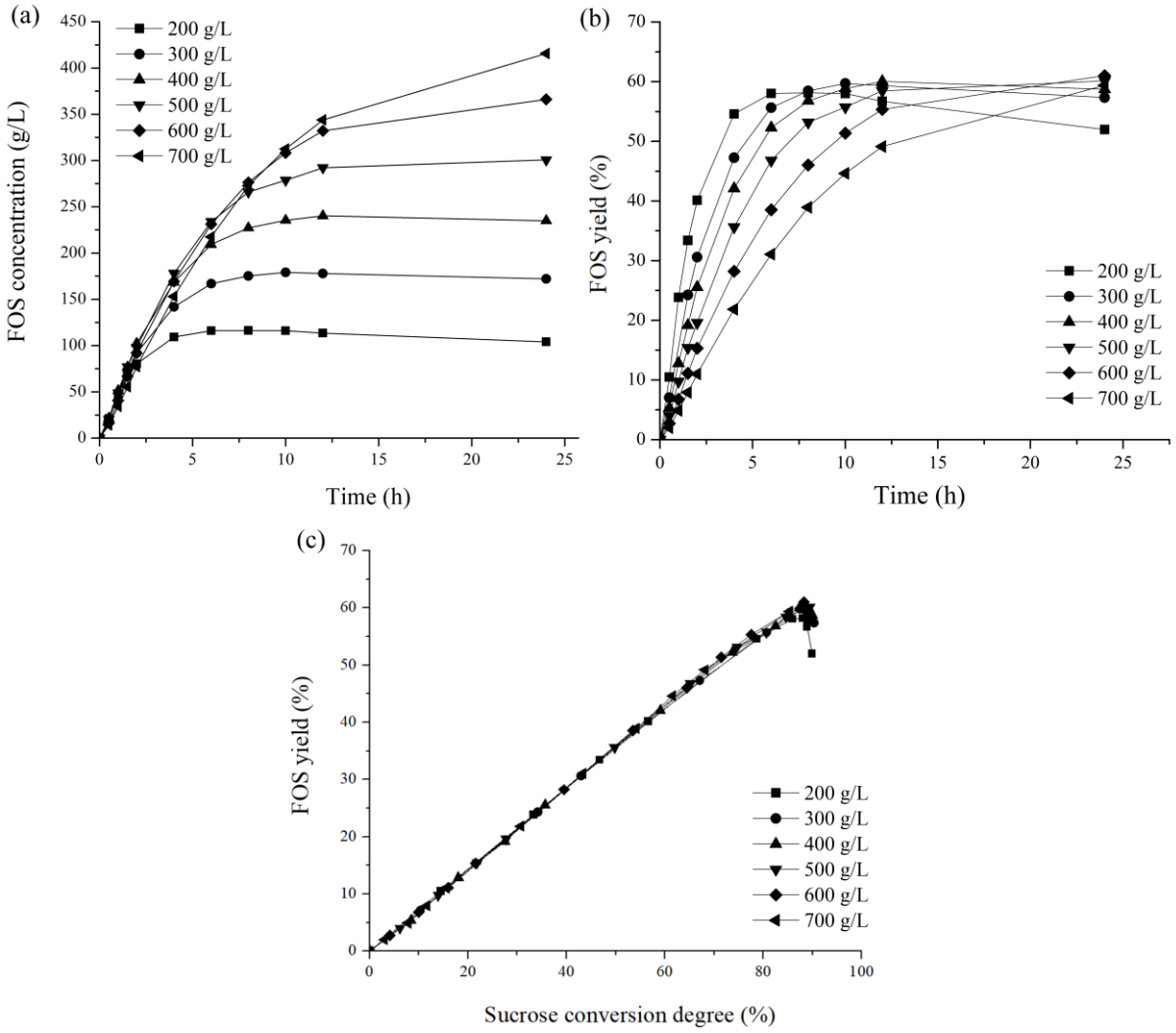


Figure 5. Effect of the sucrose concentration on FOS production: (a) FOS concentration and (b) FOS yield. (c) Dependence of sucrose conversion and FOS yield. Experiments were performed in different sucrose solution (200-700 g/L) prepared with distilled water and 1% (v/v) enzymatic preparation Pectinex® Ultra SP-L at 50 °C

blished studies (Hang & Woodams, 1996; Vega & Zniga-Hansen, 2011; Kashyap et al., 2015), which explain this phenomena by the presence of more molecules accessible for conversion to FOS at higher sucrose concentrations. Namely, more molecules can act as acceptors of the fructosyl moiety, thus suppressing potential hydrolytic activity of the enzyme and shifting reaction equilibrium towards FOS synthesis. It can be also seen that the maximum concentrations in each experiment are reached at different times. Namely, the higher initial concentration of substrate needs longer reaction times for achieving maximum concentrations, even though they stay constant for long period afterwards. At this time, although FOS concentration seems to be the same, the constant re-combination of obtained FOS molecules occurs and the ratio of individual fractions (FOS3, FOS4 and FOS5) is constantly changing. At the beginning FOS3 represents the major component of the system, while with longer times the higher FOS take precedence (Fig. 2). Therefore, to provide a mixture of the best possible prebiotic characteristics, reaction should be terminated as soon as reaching maximum concentration. When taking into consideration the total FOS yields, all analyzed reactions showed the same trend. The obtained data suggest that reaction equilibrium is attained after obtaining 60% FOS in total carbohydrates (Fig. 5b). These results are important stating that achieved FOS yields are independent of initial sucrose concentration (within the examined range).

Additionally, when these results are presented concerning the amount of converted substrate-sucrose (Fig. 5c), it can be seen that best results are achieved upon conversion of approximately 85% sucrose in each of the examined experiments. In the next period, the hydrolytic activity of the enzyme predominates, most probably leading to hydrolysis of sucrose and newly formed fructo-oligosaccharides (FOS) and generation of glucose and fructose. One of the possible explanations for existence of this equilibrium concentration (60% FOS in total carbohydrates) is that glucose formed during the reaction acts as a competitive inhibitor of FOS synthesis (Kashyap et al., 2015). Accordingly, it can be concluded that in all samples when reaction is terminated at deffined time (for each concentration), the obtained ratio of the components will be almost constant: 15% sucrose, 60% FOS, 20% glucose and up to 5% fructose. This consistency is important when planning future experiments and deciding which product are acceptable for sucrose transformation, bearing in mind the potential product must meet the minimum concentration requirements for being declared as functional.

Taking into account the foregoing, and the possibility that high concentrations could lead to sucrose crystallization that may represent severe obstacle for developing the continuous reactor systems, the concentration 500 g/L was chosen as the best suited concentration for further sucrose transformation experiments, which is consistent with published literature data (Kashyap et al., 2015). At this concentration, the value of the maximum FOS yield achieved after 24 h is 60% in total carbohydrates (300.7 g/L).

CONCLUSIONS

In the present study, the effect of key reaction conditions (temperature, pH, enzyme and sucrose concentration and reaction time) was studied in terms of achieving high FOS yields catalyzed by FTase from A. aculeatus from commercial multi-enzyme preparation Pectinex® Ultra SP-L. It was concluded that the temperature and pH, although they have a high impact on the initial reaction rates, have negligible influence on FOS synthesis. On the other hand, enzyme and substrate concentration in correlation with reaction time provides a rather significant contribution to the enhancement of FOS synthesis efficiency. Namely, after taking into account high achieved FOS yields, prerequisites for future experiments (complex reaction mixtures, different reactor configurations) and finally economic factors, it was concluded that reaction should take place in aqueous sucrose solutions (500 g/L) using 1% (v/v) of Pectinex® Ultra SP-L at 50 ºC for 24h.

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ENZIMSKA SINTEZA FRUKTO-OLIGOSAHARIDA UPOTREBOM PECTINEX® ULTRA SP-L: ISPITIVANJE UTICAJA EKSPERIMENTALNIH USLOVA

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Sažetak: Razvijanje svesti o brojnim prednostima upotrebe prebiotika kao funkcionalnih sastojaka prehrambenih proizvoda i hrane za životinje rezultira povećanjem potražnje, a samim tim i potrebom za povećanjem proizvodnje prebiotika na industrijskom novou. Ovo dodatno zahteva i razvijanje efikasnijih i ekonomičnijih procesa proizvodnje prebiotika. Cilj ovog rada bila je sinteza potvrđenih prebiotika - frukto-oligosaharida (FOS), transformacijom saharoze. Ova reakcija je odabrana prvenstveno sa aspekta mogućnosti sinteze oligosaharida manjih molarnih masa sa izraženijim prebiotskim efektom koji se ovim putem dobijaju. Kao izvor enzima korišćena je komercijalna enzimska smeša Pectinex® Ultra SP-L, čijom se upotrebom zbog niske cene na tržištu, dodatno utiče na ekonomičnost procesa. Ispitivanjem uticaja reakcionih uslova poput pH, temperature, koncentracije enzima i supstrata utvrđeno je da se variranjem uslova, kao i dužine trajanja procesa može značajno prilagoditi sastav dobijene smeše FOS. Stoga, utvrđeno je da se izvođenjem reakcije u vodenoj sredini (pH 7), na temperaturi od 50 °C upotrebom konentracije enzima od 1% (v/v) i koncentracije saharoze od 500 g/L može ostvariti maksimalni prinos FOS od 60% u ukupnim šećerima. Ovako dobijen sirup sa visokim udelom FOS se može koristiti kao aditiv proizvodima, dok se sa druge strane razvijeni proces može iskoristiti za direktnu transformaciju prehrambenih proizvoda (poput sokova, džema, nadeva, bombona, kolača i dr.) u kojima dominira saharoza kao osnovni izvor šećera, stvarajući na taj način proizvode sa manjom kalorijskom, a većom funkcionalnom vrednošću.

Ključne reči: *frukto-oligosaharidi, prebiotici, fruktoziltransferaza, enzimska sinteza*

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