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Effects of Organic Solvents on Acceptor Reactions for Oligosaccharide Synthesis Catalyzed by Glucansucrase URE 13-300

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Abstract

Background and Objective: Glucansucrases from GH70 family are effective transglucosylases, able to use non-carbohydrate acceptors. Glycosylation of flavonoids or terpenoids increases their water-solubility and bioavailability. Enzymatic glycosylation by glucansucrases can be improved by addition of organic solvents to the reaction media. Thus, the aim of the study was to assess effects of menthol, carvacrol and thymol solubilized in organic solvents on the activity of glucansucrase URE 13-300 and transferase reaction.

Material and Methods: Several organic solvents were assessed for their effects on glucansucrase activity using DNS method. Kinetic parameters in presence of the most appropriate solvents were evaluated as well. Thymol, carvacrol and menthol were solubilized in DMSO and their effects on the enzyme activity was assessed. Dynamic of oligosaccharides synthesis in aqueous-organic media was investigated using high-performance liquid chromatography.

Results and Conclusion: Maltose-derived oligosaccharides synthesized by glucansucrase URE 13-300 showed degrees of polymerization from 3 to 6 in presence of organic solvents, as well as in presence of buffer alone. Their concentrations did not differ significantly in each of the reactions in aqueous-organic media. Furthermore, kinetic parameters showed adjacent K_m values with 5% solvents compared to the control reaction in buffer. These findings revealed that the overall synthesis of glucooligosaccharides was not altered by the organic solvents, nevertheless they changed the product distribution throughout the transferase reactions. These moderate effects of the selected organic solvents were important requirement for the glycosylation of biologically active compounds for use in the food industry.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

Glucansucrases (GSs) are classified within the glycoside hydrolase family 70 (GH70) and a small fraction of them has biochemically been characterized [1]. A majority of GSs are produced by lactic acid bacteria (LAB) of *Leuconostoc*, *Streptococcus*, *Lactobacillus* and *Weissella* species [2]. The GSs and branching sucrases (BRSs) are effective as transglucosylases. Moreover, GSs use sucrose as a donor of glycosyl units for the formation of high molar mass α-glucans and BRSs introduce the glycosyl moieties as branches in linear α-1,6-linked dextrans, using it as an acceptor [3]. The GSs and BRSs are known for their diverse carbohydrate and non-carbohydrate, hydroxylated acceptors promiscuity and thus demonstrating the ability to produce α-glucooligosaccharides. Depending on the enzyme selectivity, α-glucans and the respective oligosaccharides are classified as dextrans (comprised of majorly α-1,6 linkages), alternans (alternating α-1,6 and α-1,3 linkages), reuterans (α-1,6 and α-1,4 linkages) and mutans (insoluble, majorly α-1,3 linkages) [4]. Branched dextran polymers with various ratios of α-1,2, α-1,3 or/and α-1,4 linkages are broadly studied and incorporated in food, healthcare or cosmetic uses [5,6].

In several occasions, products of transglucosylation are oligosaccharides with branched structure and demonstrate prebiotic characteristics [7,8]. Various studies have shown that maltose is the best of wide range of acceptors and the quantity of D-glucose transferred to the acceptor depends on the acceptor:donor ratio [8-10]. Use of organic solvents in glucan synthesis and/or glycosyltransferase reactions brings

advantages in various cases. Some non-conventional acceptors are poorly soluble in water; however, their solubility increases with addition of organic solvents [11,12]. Presence of water-miscible organic solvents in the reaction mixture can alter selectivity between the transferase and hydrolase activities [13].

Enzymatic glycosylation of poorly soluble acceptors such as phenolic and terpenoid compounds can overcome issues as low bioavailability and solubility when they are targeted for incorporation of various products for the food, cosmetic and pharmaceutical industries. Therefore, addition of organic solvents increases their solubility and consequent glycolsylation. Essential oil constituents are interesting objects for the glycosylation because of their diverse bioactivities. Two promising components of essential oils generally recognized as safe by the Food and Drug Administration and currently used in the food industry for flavoring and packaging for food preservation include carvacrol and thymol derived from oregano and thyme. Their combination with conventional antibiotics may provide alternative methods to overcome the problem of bacterial resistance in foods and diseases. For example, antibacterial and antibiofilm activities of the essential oils, including carvacrol and thymol, were assessed in combination with tetracycline against oral bacteria with selective antimicrobial activities [14]. High-molecular glucansucrase URE 13-300 was isolated from *Leuconostoc mesenteroides* URE 13 and then cloned in *Escherichia coli* BL21 [8]. Glucansucrase 13-300 synthes-izes an insoluble α-glucan with 67.2% of α-1,6 linkages in the major polysaccharide chain, 16.18% of α-1,3 linkages in the branching points and 8.3% of α-1,6 linkages in the branches [15]. Transferase reaction in presence of maltose and other acceptor oligosaccharide acceptors is well studied. Various initial acceptor:donor ratios result in the synthesis of oligosaccharides with degrees of polymerization (DP), ranging from DP3 to DP7 [8]. The aim of the present study was to study effects of essential oil constituents of menthol, carvacrol and thymol on the effectiveness of transferase reaction by glucansucrase URE 13-300 in presence of maltose as acceptor. The GS activity and kinetic parameters of transferase reaction in presence of organic solvents were initially investigated. In addition, effects on glucansucrase URE 13-300 activity was evaluated as well.

2. Materials and Methods

2.1. Bacterial strain

In this study, recombinant strain of *E. coli* BL21 URE 13-300 expressing glucansucrase URE 13-300 from *L. mesenteroides* URE 13 was used for the enzyme production. This was provided by the Department of Biochemistry and Microbiology, Plovdiv University, Plovdiv, Bulgaria [8].

2.2. Preparation of glucansucrase

Cultivation of *E. coli* BL21 URE-13-300 for GS production was carried out as described by Bivolarski et al. [8]. Collected cells were diluted in 20 mM of sodium acetate buffer (pH 5.3) and disrupted in Nano DeBEE high-pressure homogenizer (BEE International, MA, USA). Cell debris was removed by centrifugation at 8000× g for 15 min and the supernatant was used as a source of GS.

2.3. Glucansucrase activity assay

Effects of organic solvents on enzyme activity were assessed during enzyme incubation with various concentrations of ethanol, isopropanol, n-butanol, isoamyl alcohol, octanol, DMSO, n-hexane, acetonitrile and ethyl acetate, ranging 5-45% (v v^{-1}), in a solution with 20 mM of sodium acetate buffer (pH 5.3). One unit of GS activity is defined as the release of 1 μmol fructose per minute at 30 °C in 20 mM of sodium acetate buffer (pH 5.3), 0.05 g l^{-1} $CaCl_2$ and 100 g l^{-1} sucrose. Enzyme activity was evaluated by estimating the fructose concentration using 3,5-dinitrosalicilic acid method [16]. The pH has not been adjusted after adding the organic solvents. Addition up to 20% organic solvents did not significantly alter the reaction pH (from 5.3 to 5.5) and GS activity was in the optimum range. Effects of organic solvents were assessed by calculating percentage of inhibition, compared to the control reaction without organic solvents set as 100% activity [17]. Each reaction was set in duplicate and results were shown with standard deviation (±SD).

2.4. Glucansucrase activity assay in presence of carvacrol, thymol and menthol

Effects of essential oil components of carvacrol, thymol and menthol on glucansucrase URE 13-300 enzyme activity was assessed during enzyme incubation with growing concentrations of 10, 25, 40, 50 and 80 mM solubilized in 20% of DMSO. As described in Subchapter 2.3., 20 mM of sodium acetate buffer with pH 5.3 was used for the enzyme activity assay with 100 g l^{-1} sucrose as substrate. Enzyme activity was reassessed by estimating the fructose concentration using 3,5-dinitrosalicilic acid method [16]. The pH has not been adjusted after adding the organic solvents. The addition up to 20% did not significantly alter the reaction pH (from 5.3 to 5.5) and it was in the optimum range for glucansucrase activity. Effects of carvacrol, thymol and menthol were assessed by calculating inhibition percent, compared to the control reaction with sucrose alone as substrate and 20% of DMSO as cosolvent, set as 100% activity. Each reaction was carried out in duplicate and results were shown with ±SD.

2.5. Kinetic parameters

Effects of substrate concentrations on the rate of acceptor reactions with maltose and sucrose at an acceptor: donor ratio of 0.5 were assessed. Sucrose concentrations varied 20-800 mM and constant conditions as stated in the previous section.

In these experiments, the following concentrations of organic solvents were used, 5% of ethanol, 5% of DMSO, 5% of n-hexane, 5% of octanol, 20% of DMSO, 20% of n-hexane or 20% of octanol (v v^{-1}). Released fructose was assessed using 3,5-dinitrosalicilic acid method [16]. Michaelis constant (K_m) and maximum velocity (V_{max}) were assessed using Michaelis-Menten equation and non-linear regression approach as described previously [18].

2.6. Preparation and analysis of the enzyme reactions with maltose

Eight various acceptor reactions were set. In all of these reactions, concentrations of sucrose and maltose were 100 and 50 g l^{-1}, respectively. Reaction mixtures contained 20 mM of sodium acetate buffer (pH 5.3), 0.1% (w v^{-1}) sodium azide and organic solvent of 5% of ethanol, 5% of DMSO, 5% of n-hexane, 5% of octanol, 20% of DMSO, 20% of n-hexane or 20% of octanol (v v^{-1}). The last reaction flask included no organic solvents used as control. Each reaction initiated by adding 0.5 U ml glucansucrase URE 13-300 and samples were collected at a specific interval of time to evaluate dynamics of the transferase reaction. Reaction samples were treated with two volumes of 96% ethanol for the precipitation of glucan. Glucan was separated from the samples by centrifugation at 8000× g. Supernatant fractions, containing synthesized glucooligosaccharides (GOSs), were evaporated under vacuum to the initial volume. Assessments of the type and quantity of the synthesized GOSs, residual substrates and acceptor concentrations were carried out using high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan) with RI detector and 250 × 4.6 mm HPLC column of YMC Pack Polyamine II (YMCTM, Japan) and specific conditions as follows. Column temperature of 30 °C, flow rate of 1.0 ml/min and acetonitrile:water mobile phase of 60:40 (v v^{-1}). Results were achieved using LabSolutions chromatographic software (Shimadzu, Kyoto, Japan) against appropriate carbohydrate standard solutions.

2.7. Statistical analysis

In all experiments, Microsoft Excel and statistical software package SigmaPlot v.12.0 (Systat Software, CA, USA) were used for data analysis. All experiments were carried out in duplicate and results were present with ±SD.

3. Results and Discussion

3.1. Effects of organic solvents on the activity of glucansucrase URE 13-300

Effects of nine organic solvents on the activity of glucansucrase URE 13-300 were assessed (Table 1). Experiments were carried out until reaching more than 50% decrease of GS activity against the activity in the control reaction, set as 100%. Of the alcohol solvents, addition of 5% of n-butanol or isoamyl alcohol led to nearly complete loss of activity at 5% concentrations (99.5 and 98.5%, respectively). Drastic inhibitory effects were observed in reactions, containing 5% of ethyl acetate and 5% of acetonitrile. Isopropanol and ethanol showed moderate effects with more than 50% of decreased activity at 10 and 20% concentrations, respect-ively. Experiments with octanol showed inhibitory effects at low concentrations; however, these were not shown at concentrations greater than 20%. This might be caused by the partition between the water and organic phase, which limited enzyme interaction with the solvent. The GS activity in presence of DMSO decreased gradually; where, the enzyme preserved nearly half of its total activity at 50% concentration. The addition of DMSO to the reaction media did not result in changes in the DNS color. Contrary to the present results, inhibitory effects of DMSO on dextransucrase from *Leuconostoc mesenteroides* B-512F and alternansucrase from *L. mesenteroides* B-23192 were distinguishably higher, reaching more than 60% decrease in the enzyme activity at 40% organic phase [19]. Thus, glucansucrase URE 13-300 was less affected by DMSO in the enzymatic reaction. Ming Miao et al. reported similar results regarding DMSO and n-hexane; where, GS activity was relatively high. In general, GS from *Lactobacillus reuteri* SK24.003 seemed more active at high concentrations of ethanol, isopropanol and n-butanol, in contrast with the present findings [17]. Activity of GSs in aqueous-organic media depends on the conformation of the active site and the nature of the enzyme natural producer [12]. Recombinantly expressed glucansucrase URE 13-300 was strongly inhibited by low-molecular-weight alcohols, compared to the native enzymes. Activity of glucansucrase URE 13-300 was not modified by n-hexane in all the experiments. It is noteworthy that the enzyme structure has not been assessed in presence of the organic solvents, as glucansucrase URE 13-300 was difficult for crystallization. To the best of the authors' knowledge, there is no high-molecular-weight GS crystallized in non truncated form. Out of the organic solvents, DMSO, n-hexane and octanol showed the minimum inhibitory effects with low concentrations of ethanol. Therefore, their effects on the transferase reaction were further evaluated.

3.2. Effects of various organic solvents on transferase reactions in presence of maltose as acceptor

Considering the initial analysis of glucansucrase URE 13-300 activity in presence of various organic solvents, four of them were selected for assessing their effects on the transferase reaction, including DMSO, n-octanol, n-hexane and ethanol in two concentrations of 5 and 20%. Effects of the initial concentrations of sucrose and maltose on the acceptor reaction in aqueous media and in presence of the selected organic solvents were assessed. Enzymatic reactions with concentrations of sucrose ranging 0.028-0.730 M at a defined ratio to maltose (M/S = 0.5) were carried out. Values of K_m and V_{max} were calculated using Michaelis-Menten equation and non-linear regression approach (data not shown). Previous studies in the authors' laboratory have

established that maltose is the most appropriate acceptor for the synthesis of GOSs by transferase reaction of the studied glucansucrase URE 13-300 enzyme [8]. Enzyme activity was 3.6 U/mg, nearly 30% higher of that in 10% (292 mM) sucrose substrate alone. Affinity to sucrose substrate decreased, as the K_m value in presence of acceptor maltose increased nearly 5-fold. This result was similar to the result by Hien Pham et al., where they reported almost identical relationships [20]. The calculated K_m= 0.231 M and V_{max} = 3.6 U/mg were set as control values.

Based on the results, it was reported that addition of 5% of DMSO and n-octanol showed mild effects on V_{max} values of 3.5 and 3.3 U/mg, respectively. Similar results were shown in the K_m values of 0.216 and 0.248 M, respectively. These results correlated well with the reported inhibition of enzyme activity. However, addition of 5% n-hexane in the reaction mixture resulted in mild increases of enzyme activity with simultaneous rise in K_m value of 0.271 M sucrose. In presence of 20% DMSO, octanol and n-hexane, values of K_m increased between 13 and 26%, correlating to the inhibitory effects of those organic solvents. From previous studies carried out by the present authors, it is known that glucansucrase URE 13-300 catalyzes production of GOSs with DP 3 up to 7 with an acceptor:donor ratio of M/S≤1 [8]. Results for the enzyme activity in presence of organic solvents showed that transferase reactions could be carried out in presence of 20% concentrations of DMSO, n-hexane and octanol. It is possible that pH of the reaction media could change by the addition of higher concentrations of organic solvents as a result of the diminished aqueous content. However, when pH of the reaction media with the organic solvents was compared with the buffer, shifts in the pH value were included in the optimal range (e.g.,5.5) for glucansucrase URE 13-300.

3.3. Effects of the components from essential oils solubilized in 20% DMSO on the activity of glucansucrase URE 13-300

Activity of glucansucrase URE 13-300 was analyzed in presence of carvacrol, thymol and menthol. These compounds were selected for their diverse uses in food and pharmaceutical industries [21,22]. Final concentrations of these compounds at the reaction mixture varied 10-80 mM. In all reactions with carvacrol and thymol, no inhibition of the enzyme activity was detected. In contrast, it seemed to neutralize inhibitory effects of DMSO, which was added as a cosolvent at 20% concentration. Reactions with menthol demonstrated inhibitory effects on glucansucrase URE 13-300 by 28% at 50 mM concentrations. Similar effects were achieved while 80 mM of menthol were present in the reaction. Octanol and ethanol were assessed as cosolvents at 20% of the final concentration. In reactions where ethanol was added, decreases of the enzyme activity by 70% were observed. Menthol, carvacrol and thymol were completely soluble at 20% concentration of octanol; however, their effects in such media should further be studied.

Terpenoid compounds solubilized in DMSO could be addressed for their effective glycosylation by glucansucrase URE 13-300 due to low inhibition effects of the solvent on its activity and better solubility facilitations of the targeted compounds [20]. Such terpene alcohols could be released from their respective glycosidic precursors through enzymatic hydrolysis, resulting in controlled aroma release. In addition, anticancer, antibacterial, analgesic, anti-inflammatory activities of menthol are attractive for introducing to value-added products [23]. Recently, encapsulation of menthol and luteolin in aromatic beverage has been carried out. Production of such powders can facilitate their addition in various types of functional foods and beverages [24]. Moreover, enzyme synthesis of prebiotic oligosaccharides including glycosylated terpenoid and/or phenolic compounds is an engaging perspective.

3.4. Effects of organic solvents on the composition of GOSs, synthesized by glucansucrase URE 13-300

Assessment of the effects of these organic solvents on the synthesized oligosaccharides composition was carried out by analyzing samples at various stages of transferase reaction using HPLC (Fig. 1a, b and c). As verified by various reports, maltose is a strong acceptor of glucose units during the synthesis of GOSs by GSs, generating series of oligosaccharide products with increased DP [11]. Products of each acceptor reaction serve as acceptors for the production of GOSs with higher DP. Each new fraction, as an acceptor, is less efficient than the previous one, leading to less accumulation of GOSs with higher DP. Decreases of GOSs with DP3 during the late stages of the reaction were due to their consumption as acceptors leading to accumulation of DP4 and 5 products, which were substantially less efficient acceptors [8]. Similar functions were observed in time of the control reactions (Fig. 1c).

In the first 30 min of the performed reactions, synthesis of GOSs with DP of 3 prevailed and constituted from 25 to 27% in presence of 5% DMSO, hexane and octanol. As the reaction progressed to Hour 24, synthesis of glucooligosaccharides decreased between 20 and 28%, depending on the organic solvents (Fig. 2). This was consistent with the increased concentrations of GOSs with DP 4 to 6. Synthesized yields of oligosaccharides with DP 4 in presence of the three solvents slowly increased with 15% from 30 min to the end of the reaction, as they varied from 24 to 28 $mgml^{-1}$. The most prominent shift in dynamic of the oligosaccharide synthesis was demonstrated with products with DP 5 (Fig. 3a, b). In presence of 5% DMSO, a peak of 20 mg/ml GOSs with DP 5 at Hour 6 of the transferase reaction was seen, showing 61% increases, compared to the concentration at Minute 30). A similar peak was seen at the reaction in 5% octanol; 19 mg ml^{-1} at Hour 4 with decreases to 14.5 mg ml^{-1} at the end of the reaction (Hour 24) (Fig. 3a).

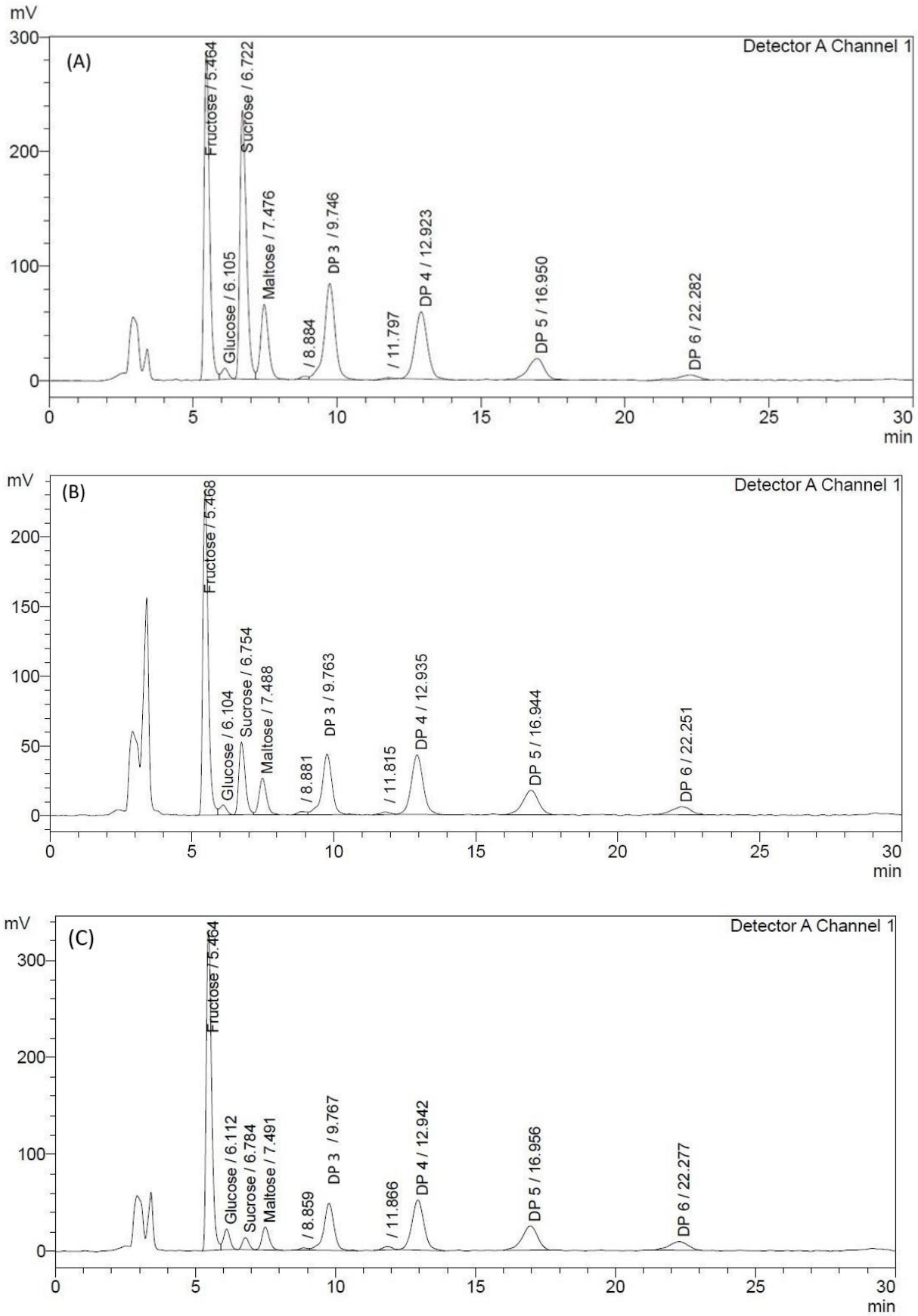

Figure 1. Profile of the oligosaccharides, synthesized in different hours of transferase reactions with maltose as an acceptor. (A): Chromatogram of the control sample, obtained at the first 30 min from the reaction initiation. (B) Chromatogram of the control sample, obtained at the first hour of oligosaccharide synthesis. (C) Chromatogram of the control sample, obtained at the end of the reaction – the 24th hour.

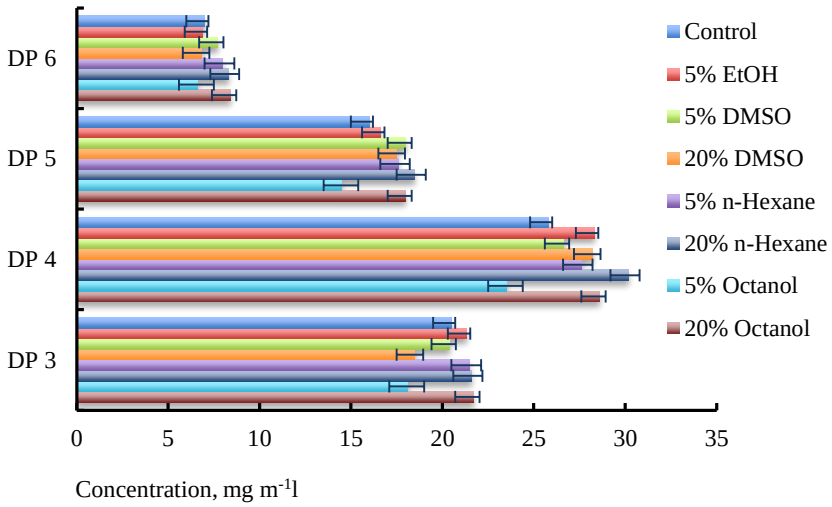

Figure 2. Overview of oligosaccharides with degree of polymerization from 3 to 6 at the 24th hour of the transferase reaction.

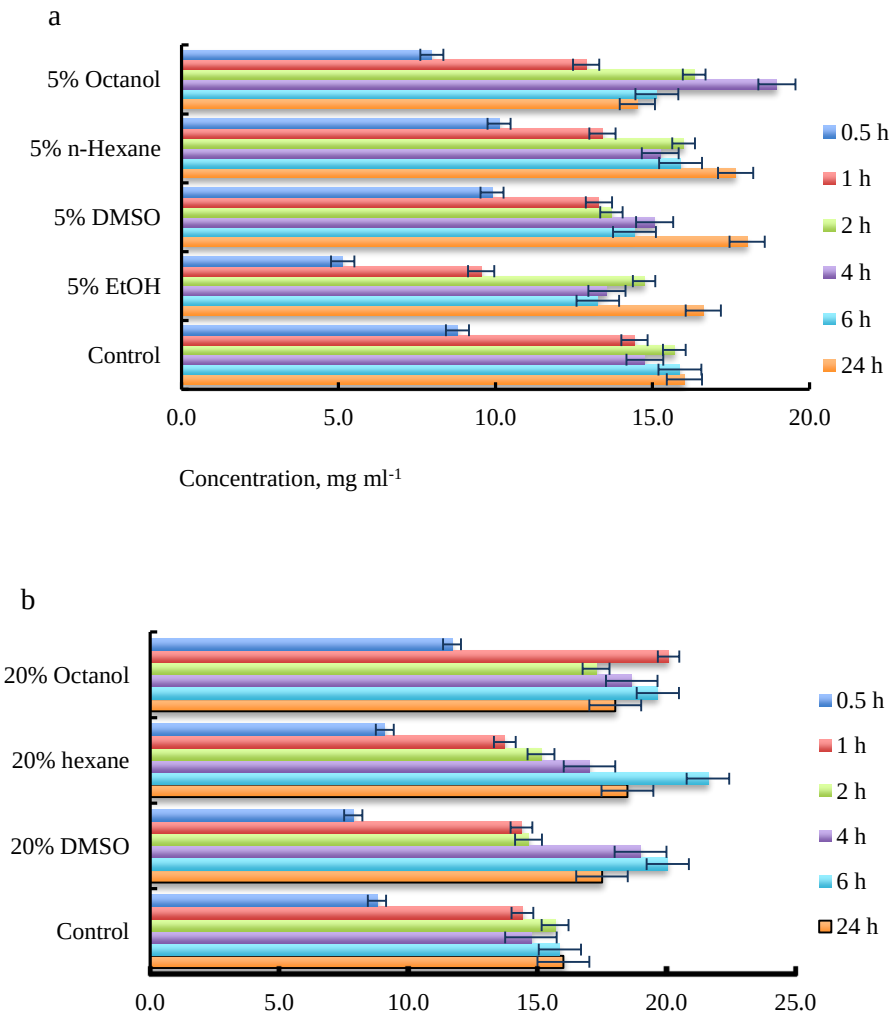

Figure 3. Synthesis of oligosaccharides with degree of polymerization of 5 during the time course of the transferase reaction. (a) Synthesis in the present of 5% organic solvents versus the control reaction. **(b)** Synthesis in the present of 20% organic solvents versus the control reaction.

Table 1. Inhibitory effects of organic solvents on the activity of glucansucrase URE 13-300

Organic solvent	Inhibition, %					
Concentration in the reaction	5%	10%	15%	20%	25%	30%
Ethanol	24.5 ± 5.2	30 ± 6.3	39.5 ± 3.9	64.6 ± 6.4	-	-
Isopropanol	41.8 ± 4.7	59.6 ± 9.6	-	-	-	-
n-Butanol	99.8 ± 1.4	-	-	-	-	-
Isoamyl alcohol	98.5 ± 1.7	-	-	-	-	-
Octanol	19.4 ± 0.9	17.7 ± 2.7	12.2 ± 5.0	8.5 ± 2.2	2.5 ± 0.4	-
DMSO	0 ± 0.2	11.2 ± 4.9	17.8 ± 3.7	19.1 ± 3.1	20.4 ± 4.2	29.6 ± 2.8
n-Hexane	0 ± 0.5	0 ± 0.3	0 ± 0.1	0 ± 0.5	-	-
Acetonitrile	77.6 ± 1.8	-	-	-	-	-
Ethyl acetate	66.7 ± 2.5	-	-	-	-	-

Oligosaccharides with DP 6 resulted in the lowest registered yield from 3 mg ml^{-1} at Minute 30 to 8 mg ml^{-1} at Hour 24. Results of GOS synthesis in presence of 20% organic solvents demonstrated similar dynamics with those at 5% concentration. In reactions with 20% DMSO at Minute 30 since reaction initiation, oligosaccharides with DP 3 at 28 mg/ml concentration were synthesized and their quantities showed 34% decrease (Fig. 4b). Oligosaccharides with DP 4, 5 and 6 increased through the reaction and a peak was recorded at Hour 6, where GOSs with DP 5 were at 20 mg/ml concentration. Similar results were achieved with 20% hexane as the maximum quantity of GOSs with DP 5 was detected at Hour 6, 21.6 mg ml^{-1} with decreases to 18.5 mg/ml at the end of the reaction. Regarding acceptor reaction in 20% octanol supplemented media, another peak of 20 mg/ml DP 5 oligosaccharides at Hour 6 was reported. Mild decreases to 18 mg ml^{-1} occurred until the end of the reaction. Independent of DMSO concentration directed the reaction towards synthesis of oligosaccharides with DP 5 (Fig. 4a,b). Products with DP 6 increased with 10%; however, they included the minor fraction in all samples. Similar findings were reported for other carbohydrate active enzymes, where an increased specificity toward particular oligosaccharide products was reported [25,26].

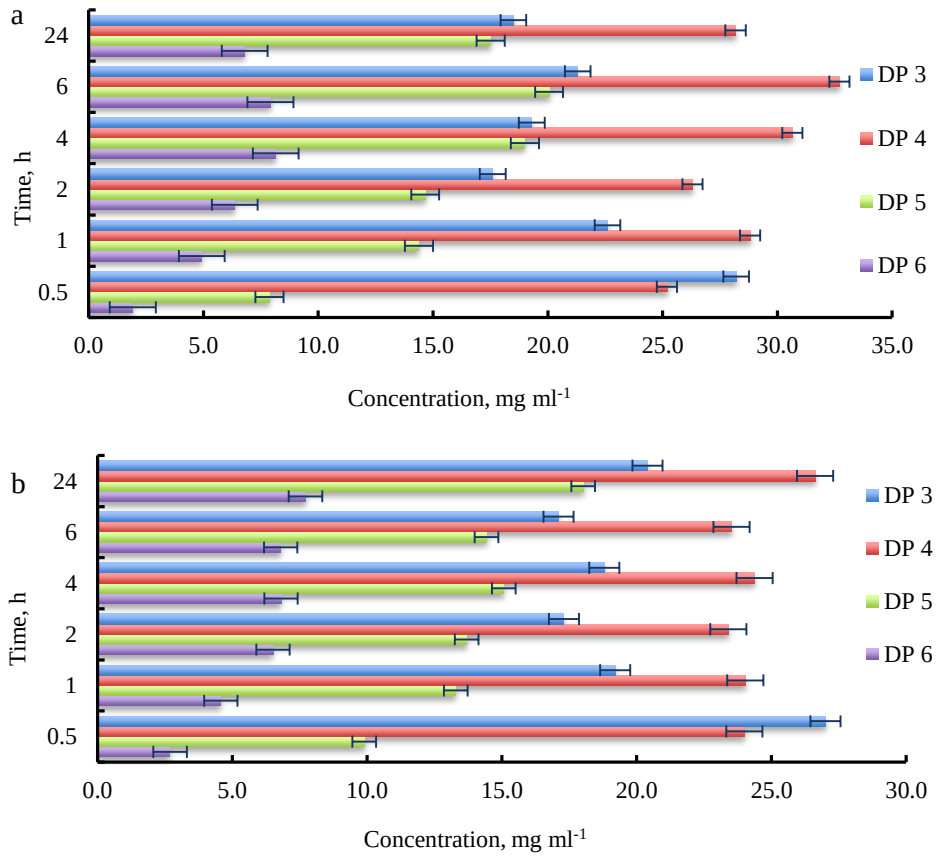

Figure 4. Dynamic changes of transferase reaction with acceptor maltose in aqueous-organic media, containing (a) 5% dimethyl sulfoxide and (b) 20% dimethyl sulfoxide

As recently reported, flavonoid glycosylation of 5 mM flavonoids dissolved in 10% DMSO was carried out [27]. In addition, biotransformation of luteolin was efficiently achieved in 20% DMSO solvent system, resulting in conversion rate of 96% luteolin glucosides [28]. In general, HPLC analysis of the synthesized oligosaccharides demonstrated that addition of DMSO up to 20% did not impair the enzyme ability to synthesize the full range of DP, shown from the control reaction.

Effects of low concentration of octanol showed decreases of all oligosaccharides, which correlated well with the inhibition towards the total enzyme activity. In contrast, the same organic solvent at 20% concentration improved all the glucooligosaccharide products. The present results indicated that oligosaccharide production throughout the transferase reaction was not affected significantly in presence of 5 or 20% DMSO, n-hexane, and octanol. Oligosaccharide synthesis with DP from 3 to 6 was verified in these reactions as well as in the control reaction without organic solvents. Therefore, inhibition effects of these solvents did not modify the acceptor reaction effectiveness. Solvents such as n-hexane and octanol are not water-miscible; however, their presence in the reaction media resulted in recomposition of the oligosaccharide mixture. The initial low concentrations of these solvents formed drops in the aqueous phase. Furthermore, oligosaccharide synthesis was carried out while continuous agitation; thus, interactions between the enzyme molecules and the solvent was achieved. The aqueous-organic media, containing water-immiscible organic solvent, was successfully used in glycosylation of aliphatic alcohols, monoterpenoids, aromatic alcohols and phenols. Some of the advantages of biphasic systems are improved enzyme stability and easier product recovery, avoiding substrate and/or product inhibition [29]. Furthermore, Devlamynck et al. carried out monoglycosylation of octanol, using wild-type and mutant variants of Gtf180-ΔN glucansucrase from *Lactobacillus reuteri* 180. Octanol was incubated with sucrose as donor of glycosyl units and yielded from 5 to 24% octyl α-D-glucosides from the wild-type and mutant variant, respectively [30].

Therefore, insights for the acceptor reactions with glucansucrase URE 13-300 might be advantageous for designing the product synthesis after rational or semi-rational mutations of some key amino acid residues [31,32]. Detailed studies on the prebiotic characteristics of GOSs, synthesized by glucansucrase URE 13-300 demonstrated that the oligosaccharide products carry prebiotic potential [8]. With modification of acceptor: donor ratio, addition of various organic solvents to the transferase reaction could be verified for further uses in new products with desirable characteristics. Further studies are currently carried out in the authors' laboratories for discovering glycosylation of non-carbohydrate acceptors with novel characteristics.

4. Conclusion

This study has shown for the first time effects of organic solvents and terpenoid compounds on glucansucrase URE 13-300 enzyme activity during transferase reaction. Organic solvents of DMSO, n-hexane and octanol have demonstrated relatively low inhibitory effects up to 20% concentration. Some of these solvents affected the oligosaccharide synthesis during the transferase reaction towards products with higher degrees of polymerization. Moreover, they do not inhibit the transferase reaction for the production of oligosaccharides with potential prebiotic effects. Thus, glycosylation of acceptor substrates with low bioavailability is possible. Glycosylated derivatives of essential oils and their compounds such as menthol can be used in design of added-value food supplements by combining gut health characteristics of prebiotic oligosaccharides with anti-microbial, analgetic and anti-inflammatory characteristics of these compounds.

5. Acknowledgements

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6. Conflict of Interest

The authors report no conflict of interest.

7. Authors Contributions

All authors contributed to this study. Conceptualization, S.A. and I.I.; methodology, S.A., T.V., V.B. and I.I.; software, S.A.; validation, T.V., V.B. and I.I.; formal analysis, S.A., T.V. and V.B.; investigation, S.A. and I.I.; resources, S.A.; data curation I.I., T.V. and V.B.; writing-original draft preparation, S.A.; writing-review and editing, T.V., V.B. and I.I.; visualization, S.A.; supervision, I.I. and T.V.; project administration, I.I.; funding acquisition, I.I. All authors have read and verified published version of the manuscript.

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اثر حلال‌های آلی بر واکنش‌های پذیرنده برای تولید الیگوساکارید کاتالیز شده توسط گلوکان سوکرآز URE 13-300

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واژگان کلیدی
- خانواده GH70
- گلیکواولیگوساکاریدها
- کاتالیز ناهمگن
- ترانس گلیکوزیلاسیون

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چکیده

سابقه و هدف: گلوکان سوکرازهای خانواده GH70 ترانس گلوکوزیلازهای موثری هستند که می‌توانند از پذیرنده‌های غیر کربوهیدراتی استفاده کنند. گلیکوزیلاسیون فلاونوئیدها یا ترپنوئیدها حلالیت در آب و فراهمی زیستی آنها را افزایش می‌دهد. گلیکوزیلاسیون آنزیمی توسط گلوکان سوکرآزها را می‌توان با افزودن حلال‌های آلی به محیط واکنش بهبود بخشید. بنابراین، هدف از این مطالعه بررسی اثرات منتول، کارواکرول و تیمول محلول در حلال‌های آلی بر فعالیت گلوکان سوکراز URE 13-300 و واکنش ترانسفراز بود.

مواد و روش ها: چندین حلال آلی از نظر اثر آنها بر فعالیت گلوکان سوکرآز با استفاده از روش DNS[۱] مورد بررسی قرار گرفت. پارامترهای سینتیک در حضور مناسب‌ترین حلال‌ها نیز مورد ارزیابی قرار گرفت. تیمول، کارواکرول و منتول در DMSO[۲] حل شدند و اثرات آنها بر فعالیت آنزیم مورد بررسی قرار گرفت. دینامیک تولید الیگوساکاریدها در محیط های آبی-آلی با استفاده از کروماتوگرافی مایع با کارایی بالا مورد بررسی قرار گرفت.

یافته‌ها و نتیجه‌گیری: الیگوساکاریدهای مشتق از مالتوز و تولید شده توسط گلوکان سوکرآز URE 13-300 در حضور حلالهای آلی و همچنین در حضور بافر به تنهایی، درجات پلیمریزاسیون از ۳ تا ۶ را نشان دادند. غلظت آنها در هر یک از واکنش‌ها در محیط‌های آبی-آلی تفاوت معنی‌داری نداشت. علاوه بر این، پارامترهای سینتیک مجاور K_m با ۵ درصد حلال قابل مقایسه با واکنش کنترل یا شاهد در بافر بود.این یافته‌ها نشان داد که تولید کل گلوکولیگوساکاریدها توسط حلال‌های آلی تغییر نمی‌کند، با این وجود، آنها توزیع محصول را در سراسر واکنش‌های ترانسفراز تغییر دادند. این اثرات ملایم حلال‌های آلی انتخابی، برای گلیکوزیلاسیون ترکیبات فعال بیولوژیکی برای استفاده در صنایع غذایی مهم بود.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

[۱] The dinitrosalicylic acid (DNS) method
[۲] Dimethyl sulfoxide (DMSO)