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Oligosaccharide Synthesis in Fruit Juice Concentrates Using a Glucansucrase From *Lactobacillus reuteri* 180

Simon Johansson[a,b], *Bernd Diehl*[c], *Paul Christakopoulos*[b], *Sean Austin*[a], *Christina Vafiadi*[a,*]

[a] *Nestlé Research Center, Vers-Chez-Les-Blanc, Lausanne, Switzerland*
[b] *Biochemical Process Engineering, Division of Chemical Engineering, Department of Civil, Environmental and Natural Resources Engineering, Luleå University of Technology, 971-87 Luleå, Sweden*
[c] *Spectral Services AG, Köln, Germany*

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ABSTRACT

The application of the glucansucrase GTF180 from *Lactobacillus reuteri* 180 in fruit juice concentrates for the synthesis of glucooligosaccharides was investigated. Reaction parameters such as temperature, pH, substrate and enzyme loading, Ca^{+2} addition and incubation time were investigated in high concentration sucrose solutions. The optimum conditions (50 °C, pH 4.5, enzyme loading 14.47 U/$g_{sucrose}$ with 1 mM $CaCl_2$, undiluted fruit juice concentrates) were applied in apple and orange juice concentrates. More than 95% of the intrinsic sucrose was converted to oligosaccharide products after 90 min. The main products were leucrose, isomaltose and isomaltotriose. The enzyme was deactivated during standard fruit juice pasteurization conditions (95 °C, 15 s). The oligosaccharides were stable during the pasteurization process, showing a good potential for industrial applications. DOSY NMR analysis of the enzymatically modified fruit juice concentrates showed that α1→6 glycosidic linkages are predominant in apple juice, while products in orange juice possess both α1→6 and α1→3 glycosidic linkages. The presence of α1→2 glycosidic linkages were also observed at a lower extent.

1. Introduction

A diet rich in fruit and vegetables is recommended as part of a healthy life-style. Current recommendations are for adults to consume five portions (about 400 g) of fruit and vegetables each day (WHO Technical Report Series, 2013) . Historically, consumption of fruit juices was recommended to contribute to these five serving per day. However, several medical associations (American Academy of Pediatrics, Canadian Health Association) are now questioning the relevance of including fruit juice as a serving of fruit per day. Their main argument is that despite containing positive nutrients such as vitamins, fruit juices are equally loaded with sugars as soft drinks. High sugar intakes, and especially in the form of beverages, has been linked with several non-communicable diseases, such as obesity, type 2 diabetes and cardiovascular diseases (Te Morenga et al., 2013). Even 100% fruit juices that contain no added sugar can have up to 140 g sugars per L (USDA, National Nutrient Database for Standard Reference Release). Therefore, technological solutions allowing the reduction of the intrinsic sugar content of fruit juices without altering its other components may represent an attractive way to help consumers increase their intake of fruit whilst meeting their demand for healthier carbohydrate and sugar profiles in foods, (Goffin et al., 2011).

* Corresponding author. Tel.:.+41 217 85 8469.
E-mail address: Christina.Vafeiadi@rdls.nestle.com (C. Vafiadi).
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Fruit juice concentrates are widely used ingredients in the food industry and are obtained after water has been physically removed from the fruit juice (Codex, 2005), to ensure longer shelf life by reducing the risk of microbial contamination. The low water content of such concentrates makes them promising substrates for the production of oligosaccharides, using enzymes with transglycosylation activities, which are favoured versus hydrolysis under such conditions.

Glucosyltransferases or glucansucrases (GTF) are transglycosidase enzymes (EC 2.4.1.-) using sucrose as substrate to synthesize glucoconjugates, oligosaccharides and polysaccharides using the energy of the glycosidic bond of sucrose to fuel the reaction (Monsan et al., 2000, Monsan et al., 2001). Glucansucrases can catalyze three types of reactions; polymerization, hydrolysis and an acceptor reaction. Catalysis by glucansucrases is a two-step reaction, which starts with sucrose binding to the active site of the enzyme, followed by the cleavage of the α1↔2 glycosidic linkage between glucose and fructose yielding a covalent glycosyl-enzyme intermediate and a liberated fructose molecule. It is the nature of the acceptor substrate which determines the reaction that will occur. The simplest reaction uses water as an acceptor and results in the hydrolysis of sucrose into glucose and fructose. Alternatively, the glycosyl intermediate can be transferred to a growing α-glucan chain in the polymerization reaction (Leemhuis et al., 2012). The glycosyl moiety from sucrose can also be transferred to an acceptor molecule other than water (e.g. glucose, fructose, maltose and a non-carbohydrate acceptor) instead of the growing polymer chain in a so-called acceptor reaction yielding either an sucrose isomer, an oligosaccharide or a glucoconjugate (Demuth et al., 2000; Monsan et al., 2001). A well-known product from the acceptor reaction is leucrose, a sucrose isomer which is formed when fructose acts as acceptor, forming an α1→5 glycosidic linkage (Leemhuis et al., 2012).

Leemhuis et al. (2013) gave an insight in the understanding of the mechanism of α-glucan polymer formation based on the elucidated three-dimensional structures of glucansucrase enzymes. *L. reuteri* 180 GTF180 glucansucrase has been biochemically and structurally characterized (Pijning et al., 2008), showing promising characteristics for application at acidic pHs, (pH range of fruit juices) and forming products with α1→6 and α1→3 linkages, which are more slowly or less digestible than α1→4 *in vitro* (Hodoniczky et al., 2012, Nguyen et al., 2014). A more recent study on the crystal structure of the truncated *L. reuteri* 180 GTF180 (Meng et al., 2015a), showed the importance of the domain V of the enzyme in the formation of polysaccharides (processive mechanism, presence of domain V) versus the formation of oligosaccharides (non-processive mechanism, absence of domain V). In the same study, mutations of the truncated GTF180 are also proposed to restore polysaccharides synthesis. These information are important to select the appropriate biocatalysts for the synthesis of the desired oligosaccharide or polysaccharide products for different applications. Moreover, an interesting study on the application of the *L. reuteri* 121 GTFA glucansucrase illustrated the impact of sucrose initial concentration on the ratio of oligosaccharide versus polysaccharide synthesis (Meng et al., 2015b).

In the present study, the potential use of the glucansucrase GTF180 from *L. reuteri* in fruit juice concentrates for the synthesis of glucooligosaccharides was evaluated. Reaction parameters such as temperature, substrate and enzyme loading, Ca^{+2} addition and incubation time were investigated in highly concentrated sucrose solutions. The relative amounts of different glycosidic linkages in the oligosaccharides produced in apple and orange juice concentrates were determined using DOSY-NMR.

2. Materials and Methods

2.1. Enzyme

The glucansucrase GTF180 from *L. reuteri* 180 was provided by Biocatalysts Ltd. The enzyme was provided in a powdered form with an activity of 2475 U/g_{powder} and a specific activity of 4.166 $U/mg_{protein}$, measured as described in Section 2.3.

2.2. Materials and chemicals

Glucose, fructose, leucrose, isomaltose, sucrose, isomaltotriose, maltose, panose, maltotriose, maltotetraose and calcium chloride were purchased from Sigma Aldrich USA. Sodium hydroxide solution (1 M) and sodium hydroxide pellets were purchased from Merck. Apple juice concentrate (60 °Bx, 2.3% acidity, pH 3.44) and orange juice concentrate (65.8 °Bx, 4.52% acidity, pH 3.55) were supplied by Austria juice; Ybbstaller fruit Austria and Argoterenas S.A- Industrial citrus, respectively.

2.3. Glucansucrase activity assay

Enzymatic activity of the glucansucrase was determined by measuring fructose release from sucrose using the DNS method. Reactions were performed by incubating the enzyme at 22 °C for 30 min in 6.5% w/v sucrose solution in phosphate-citrate buffer (100 mM, pH 4.5), in the presence of $CaCl_2$ (1 mM). One unit of enzyme activity was defined as the release of 1 μmol of fructose per min under the above described conditions. All experiments have been performed in duplicate with <10% standard error for each set of results.

2.4. Determination of total, hydrolytic and transferase activity of GTF180

Enzymatic activity of GTF180 was determined by measuring glucose and fructose release from sucrose as previously described (van Geel-Schutten et al., 1999), with a few modifications. Reactions were performed in duplicate at 50 °C in a final volume of 200 μL in 1.5 mL microtubes under agitation using an Eppendorf thermomixer. The initial sucrose concentration was 50 mM in citrate phosphate buffer at different pH values (3.0–7.0, 0.1 M) containing NaN_3 (0.04% w/w) with or without the presence of $CaCl_2$. Reactions were stopped by the addition of 0.1 M NaOH. The amount of released fructose corresponds to the total enzyme activity. The amount of released glucose represents the hydrolytic activity; the transferase activity is represented by the amount of released fructose minus the amount of released glucose. Glucose was measured using an enzymatic kit based on hexokinase and glucose-6-phosphate dehydrogenase (K-FRUGL, Megazyme). Fructose concentrations were measured using the same kit, with the additional step of phosphoglucoisomerase. One unit of enzyme activity is defined as the release of 1 μmol of monosaccharide per min, under the specified assay conditions mentioned in each case. All experiments have been performed in duplicate with <10% standard error for each set of results.