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OBTENÇÃO DE PRODUTOS TRANSGLICOSILADOS DO ESTEVIOSÍDEO E AVALIAÇÃO DE EFEITOS BIOLÓGICOS E SENSORIAIS

CASSANDRA MEIRELES TERRES RIBEIRO

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Dissertação apresentada ao programa de Pós Graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte das exigências do Programa de pós graduação em ciência de alimentos para obtenção do título de mestre em Ciência de Alimentos

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2013

Orientador(a):
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BIOGRAFIA

CASSANDRA MEIRELES TERRES RIBEIRO nasceu em 04 de dezembro de 1986, na cidade de Pelotas/RS. Possui graduação em Tecnologia em Industrialização de Carnes pela Universidade Tecnológica Federal do Paraná (UTFPR) *campus* Medianeira/PR. Possui especialização em Microbiologia Aplicada pela Universidade Estadual do Oeste do Paraná (Unioeste). Tem experiência nas áreas de ciência e tecnologia de alimentos, atuando principalmente nas áreas de Bioquímica e Microbiologia de alimentos.

Dedico

Ao meu esposo Maicom, o qual soube compreender tanta ausência, sempre me apoiando e incentivando.

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Agradeço primeiramente a Deus. A minha família, principalmente ao meu esposo Maicom Ribeiro. As amigas e colegas, Adriela Rydlewski, Márcia Anjos, Simone Hoffmann e Milena Veronezi pelo apoio, incentivo e ajuda em todos os momentos. Ao parceiro de laboratório Sérgio Dacome por toda ajuda, paciência e dedicação. A Embrapa instrumentação (São Carlos/SP). Ao laboratório de Fisiologia Humana da UEM, principalmente aos professores Drs Cecília Costa, Nilton e Márcia Brito e a todos que colaboraram no experimento in vivo. Ao departamento de farmácia, principalmente Claudio Novello pela atenção e auxílio. A professora Drª Graciette Matioli pelo empréstimo de alguns equipamentos do seu laboratório. Ao meu orientador, professor Dr Sílvio Cláudio da Costa, pelos ensinamentos, pela paciência, por tudo.

APRESENTAÇÃO

Esta dissertação de mestrado está apresentada na forma de UM artigo científico.

1 TERRES-RIBEIRO, C.M. DACOME, A.S. MATIOLI, G. BRITO, N.A. BRITO, M.R.A. MONTEIRO, A.R.G. MAREZE-COSTA, C. E. COSTA, S.C. **Obtaining transglycosylated products from stevioside and an evaluation of its biological and sensory effects** Revista Food and Bioprocess Technology.

GENERAL SUMMARY – OBTAINING TRANSGLYCOSYLATED PRODUCTS FROM STEVIOSIDE AND AN EVALUATION OF ITS BIOLOGICAL AND SENSORIAL EFFECTS

INTRODUCTION: Stevioside is one of the major glycosides in *Stevia rebaudiana* (Bert.) Bertoni leaves and is a by-product from the industrial production of stevia sweeteners due to its bitter aftertaste relative to rebaudiosides A and D. The processes for obtaining rebaudioside A generate large amounts of stevioside with a low capacity for insertion in the sweetener market. Large amounts of stevioside are even produced from selected varieties of Stevia with high rebaudioside A contents, such as Stevia UEM-320. One strategy for improving the sensory profile of stevioside is enzymatic modification (or the transglycosylation process). With this technique, the oligosaccharides that are anchored in carbons 13 and 19 are altered, which permits the formation of products that maintain their sweetening power with an improved sensory profile. In this case, CGTase from diverse groups of microorganisms were used for the intermolecular transglycosylation of stevioside.

OBJECTIVES: The objective of this study was to test the CGTase commercial enzyme (Toruzyme® - Novozymes®) for the enzymatic treatment of stevioside that is generated during the purification of rebaudioside A in the NEPRON Pilot Unit. This test was conducted to determine the conversion rates of the glycosides and to identify new glycosides. Moreover, this study sought to assess the effects of enzymatically modified products on the blood glucose levels in normal rats and to evaluate their sensory properties.

MATERIALS AND METHODS: The stevioside was obtained at the NEPRON Pilot Unit by using the methodology described by Dacome (2003). Transglycosylation experiments were performed according to the methodology described by Abelyan et al. (2004), and chromatographic analyses were conducted according to Dacome (2005). Identification of the stevioside and transglycosylated products was performed by direct insertion LC-MS with the direct insertion method. To determine the effects of the transglycosylated products on blood glucose, the recommendations of Krishnamurti and Steffes 2001; the Diabetes control group, 1998; and UKPDS, 1998) were followed. Sensory analysis was performed with 50 untrained participants by following the 9-point hedonic scale. The resulting data were submitted for an analysis of variance (ANOVA) test followed by Tukey's test ($p<0.05\%$).

RESULTS AND DISCUSSION: The natural sweetener stevioside (5.8 g) was obtained from *Stevia rebaudiana* (Bert.) Bertoni leaves with a purity of 90%. The leaves were enzymatically treated with commercial CGTase (Toruzyme) with stevioside concentrations of 0.68 to 2.75 U/g and 5.8 g of maltodextrin for 24 h. The mean stevioside concentration decreased by a rate of 52.55% with a maximum decrease of 58%. When the incubation kinetics were measured, increased incubation times resulted in the conversion of up to 70% of the stevioside. The enzymatically treated product was analyzed by HPLC/IR and HPLC/UV (210 nm). Four additional signals were detected in addition to the signal that corresponded to stevioside. LC-MS confirmed the presence of stevioside ([M+Na] 827.4) and steviol glycosides with one, two or three attached

glucose molecules (m/z 989.4, 1151.5 and 1313.4). The venous infusion of 5.6 mM of the enzymatically modified product did not significantly affect the plasma glucose concentrations in normal rats. In addition, the enzymatically treated product did not interfere with glucose tolerance. The sensory analysis revealed that the enzymatically treated product had a better aftertaste with a mean value of 6.92 (relative to 4.52) for stevioside. In addition, this product was preferred by 86% of the testers.

CONCLUSIONS: The commercial enzyme Toruzyme (CGTase), with a stevioside concentration of 0.68 to 2.75 U/g, promoted the transglycosylation of stevioside. In this case, stevioside derivatives with one, two or three attached glucose molecules were identified in the transglycosylated product. The transglycosylated product did not cause any significant effects when added at a concentration of 5.6 mM regarding the plasma glucose levels in normal rats. In addition, the transglycosylation process generated a mix of enzymatically modified products with less of a bitter aftertaste that were preferred over stevioside by the testers.

KEY WORDS: stevioside, stevia, Toruzyme, transglycosylation.

RESUMO GERAL – OBTENÇÃO DE PRODUTOS TRANSGLICOLISADOS DO ESTEVIOSÍDEO E AVALIAÇÃO DE EFEITOS BIOLÓGICOS E SENSORIAIS

INTRODUÇÃO: O esteviosídeo, um dos glicosídeos presentes em maior abundancia nas folhas de *Stevia rebaudiana* (Bert.) Bertoni, tem sido considerado atualmente como um subproduto pelas indústrias que produzem adoçantes de estévia, em função do seu gosto residual amargo ou "after taste" em relação aos rebaudiosideos A e D. Processos de obtenção do rebaudioisideo A, mesmo a partir de variedades selecionadas com alto conteúdo de rebaudiosideo A, como é caso da Stevia UEM-320, acabam gerando uma grande quantidade de esteviosídeo, com baixa capacidade de inserção no mercado de adoçantes. Uma das estratégias para melhorar o perfil sensorial do esteviosídeo é a modificação enzimática ou processo de transglicosilação, por meio do qual os oligossacarídeos ancorados nos carbonos 13 e 19 são alterados podendo gerar produtos que mantém o poder edulcorante, porém com melhoria no perfil sensorial. Para tal modificação CGTase de diversos grupos de microrganismos tem sido empregadas na transglicosilação intermolecular do esteviosídeo.

OBJETIVOS: O objetivo deste trabalho foi testar a enzima comercial CGTase (Toruzyme® - Novozymes®) para tratamento enzimático do esteviosídeo gerado nos processos de purificação do rebaudiosídeo A na Unidade Piloto do Nepron, determinar a taxa de conversão dos glicosídeos presentes e identificar os novos glicosídeos, avaliar o efeito dos produtos modificados enzimaticamente sobre a glicemia de ratos normais e as propriedades sensoriais.

MATERIAL E MÉTODOS: O esteviosídeo foi obtido na Unidade Piloto do NEPRON de acordo com metodologia descrita por Dacome (2003). Experimentos de transglicosilação foram realizados de acordo com as metodologias descritas por ABELYAN,*et al*, 2004. As análises cromatográficas foram realizadas de acordo com Dacome (2005). A identificação do esteviosídeo e dos produtos transglicosilados foram realizadas em LC-MS pelo método de inserção direta. A metodologia para determinação do efeito dos produtos transglicolisados sobre a glicemia seguiu as recomendações de KRISHNAMURTI U, STEFFES M.W, 2001. The Diabetes control group, 1993. UKPDS, 1998. E a analise sensorial foi realizada com 50 julgadores não-treinados seguindo a escala hedônica de 9 pontos e sumetidos à análise de variância (ANOVA) seguida do teste de Tukey ($p<0,05$).

RESULTADOS E DISCUSSÃO: O adoçante natural esteviosídeo (5,8 g) obtido das folhas de *Stevia rebaudiana* (Bert.) Bertoni com pureza de 90% foi tratado enzimaticamente com CGTase comercial (Toruzime) na faixa de concentração de 0,68 a 2,75U/g de esteviosídeo na presença de 5,8 g de maltodextrina por um período de 24 horas, obtendo-se uma taxa média de redução na concentração de esteviosídeo de 52,55%. A maior taxa de redução na concentração de esteviosídeo foi de 58%. Quando realizada uma cinética de incubação, aumentando este tempo obtivemos até 70% de conversão do esteviosídeo. O produto tratado enzimaticamente foi analisado por meio de CLAE/IR e CLAE/UV (210 nm), sendo detectado além do sinal correspondente ao esteviosídeo quatro sinais adicionais. Por meio de LC-MS foi confirmada no produto tratado enzimaticamente a presença do esteviosídeo ([M+Na]

827.4), e de glicosídeos do esteviol, acrescidos de uma, duas e três moléculas de glicose (m/z 989.4, 1151.5 e 1313.4) em relação ao esteviosídeo. A infusão venosa de 5,6 mM do produto modificado enzimaticamente não causou nenhum efeito significativo na concentração de glicose plasmática de ratos normais. Verificou-se ainda que o produto tratado enzimaticamante não interferiu na tolerância da glicose. A análise sensorial revelou que o produto tratado enzimaticamente apresentou gosto residual de melhor qualidade com média de 6,92 contra 4,52 para o esteviosídeo, sendo preferido por 86% dos julgadores.

CONCLUSÕES: A enzima comercial toruzyme (GCTase) na faixa de concentração de 0,68 a 2,75 U/g de esteviosídeo um foi capaz de promover a transglicosilação do esteviosídeo, tendo sido identificados no produto transglicolisado derivados do esteviosídeo acrescidos de uma, duas e três moléculas de glicose. O produto transglicosilado na concentração de 5,6 mM não causou nenhum significativo na concentração plasmática de ratos normais ($p<0,05$). O processo de transglicosilação gerou uma mistura de produtos modificados enzimaticamente com menor gosto residual amargo sendo preferido pelos julgadores em relação ao esteviosídeo.

PALAVRAS CHAVES: esteviosídeo, estévia, toruzyme, transglicosilação

Obtaining transglycosylated products from stevioside and an evaluation of its biological and sensory effects

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1. ABSTRACT

Stevioside is a by-product of industrial stevia sweetener production due to its bitter aftertaste. One strategy for improving the sensory profile of stevioside is enzymatic modification (or transglycosylation). The purpose of this study was to test cyclodextrin-glycosyltransferase (CGTase), a commercial enzyme (Toruzyme®, Novozymes®), for the enzymatic treatment of stevioside that is formed during the purification of rebaudioside A. The stevioside and transglycosylated products were identified with liquid chromatography-mass spectrometry (LC-MS), and the effects of the transglycosylated products on blood glucose were assessed in rats by venous infusion. The presence of stevioside and steviol glycosides with one, two or three glucose molecules was confirmed in the enzymatically treated product. The conversion rate of stevioside reached 70%. The modified product did not significantly affect the plasma glucose concentrations and did not interfere with glucose tolerance. Transglycosylation generated a mix of enzymatically modified products with a less bitter aftertaste than stevioside. These modified products were preferred by 86% of the testers, which suggests that they could be used as a low-calorie sweetener.

KEY WORDS: Stevioside; transglycosylation; Maltodextrin; alpha-amylase; Stevia.

2. INTRODUCTION

Stevia rebaudiana Bertoni was first described in 1899 by the naturalist Moises S. Bertoni. However, *Stevia rebaudiana* was known and used historically by native Tupi-Guaranis to sweeten beverages and prepare medicines (Bertoni, 1899).

The main components that are responsible for the intense sweetening power of stevia leaves are diterpene glycosides or steviol glycosides. Among these components, we focused on stevioside, rebaudioside C and rebaudioside A as a function of their quantity. The relative concentrations of these glycosides in the stevia leaves were dependent on the variety or clonal line of the stevia (Dacome et al., 2003).

The insertion of stevia extracts in the sweetener market has been hindered by the bitter aftertastes of stevioside and rebaudioside C. Thus, efforts have been made to obtain plants with rebaudioside A as the major component. Another strategy used to facilitate the use of stevia extracts is the enzymatic modification of stevioside, which forms transglycosylated products with better sensory profiles. The transglycosylation process changes the original structure of the oligosaccharides. Essentially, the sensory profile or the quality of the sweet taste is positively correlated with the number of monosaccharides in the oligosaccharide structure that are anchored to the steviol carbons 13 and 19 (Figure 1) (Abelyan et al., 2004).

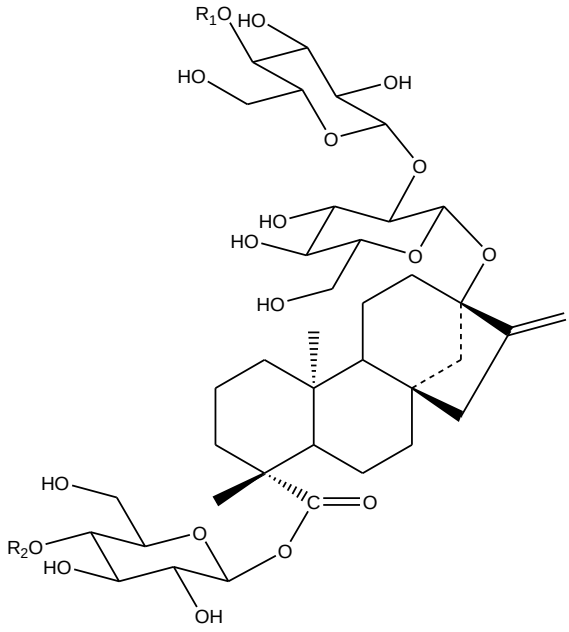


Figure 1: The Chemical structure of stevioside with two points susceptible to transglycosylation by enzymatic action (R1 and R2)

Many enzyme groups have been used to conduct transglycosylation of stevia glycosides. Among these enzymes, we highlight alpha-glycosidase, alpha-amylase, cyclodextrin-glycosyltransferase (CGTASE, EC 2.4.1.19) and dextransucrase. CGTase is a microbial enzyme that is capable of converting starch into cyclodextrins through cyclization reactions. This enzyme is also capable of performing coupling and disproportionation reactions (Jung et al., 2007; Moriwaki et al., 2009). This extracellular enzyme is produced by many types of microorganisms, such as *Thermoanaerobacterium, Klebsiella oxytoca* and many *Bacillus* species (including *B. macerans, B. circulans, B. megaterium, B. firmus, B. stearothermophilus* and *B. lentus*; Biwer et al., 2002; Moriwaki et al., 2009).

Depending on the type of enzyme employed in the transglycosylation process, starch, cyclodextrins, sucrose or maltodextrin can be used as a monosaccharide source.

3. MATERIALS AND METHODS

Stevioside was acquired in the pilot unit of the Nucleus of Natural Product Research (Núcleo de Estudos de Produtos Naturais – NEPRON) at the State University of Maringá (Universidade Estadual de Maringá - UEM). NEPRON maintains a collection of plants with different glycoside and steviol composition profiles.

Stevioside was obtained by separation during the rebaudioside A isolation process, which was conducted according to the methodology described in the literature (Dacome et al., 2003).

The cyclodextrin-glycosyltransferase (CGTase) enzyme that was used in this study was a commercial enzyme (Toruzyme®) that was provided by Novozymes® S/A with a specific activity of 26.6 U/MG.

Maltodextrin (Dextrin 10 from maize starch, article 31,410) was obtained from Fluka (Buchs, Switzerland). All other reagents were analytical grade.

Transglycosylation

For transglycosylation, 5.8 g of maltodextrin were dissolved in 50 mL of water with 5.8 g of stevia extract. For complete dissolution, the mixture was incubated at 45°C for 15 min. Next, 8 U (86 μL) of the Toruzyme enzyme were added and the pH of the medium was adjusted to 5.8 with a 0.1 M sodium phosphate buffer solution. The final volume of the reaction medium was brought to 100 mL with distilled water (Abelyan et al., 2004, with modifications).

The medium was incubated in an orbital agitator (Shaker - ACB Labor) at 120 rpm for 8 h and 45°C before incubating for 15 h at 32°C. The enzyme was inactivated by boiling the medium for 10 min. Next, the medium was filtered in a Zetaplus60 S before completely drying in a rotary evaporator (Büchi brand, model RE 120).

Analysis of the transglycosylation products by High Performance Liquid Chromatography (HPLC)

Exactly 10 mg of the powder that was obtained from the rotary evaporator was added to 2.0 mL of deionized water with 8.0 mL of acetonitrile (J.T. Baker HPLC grade and 99.9% concentration).

The mixture was filtered 10 times and was analyzed in a liquid chromatographer (Gilson brand, model 207 with UV VIS 210 nm detector) (Dacome et al., 2003).

Analysis of the transglycosylation products by Liquid Chromatography with Mass Spectrometry (LC/MS)

The samples were directly infused in acetonitrile (J.T. Baker HPLC grade 99.9% concentration) and ultrapure water (ESI-MS/MS analyzer; Waters-Micromass brand, model Quattro LC).

The acute effects of the venous infusion of transglycosylated steviosides on the blood glucose and glucose tolerance of normal rats.

The experimental protocols in this study met the ethical principles of animal experimentation and were approved by the Ethics Committee on animal experimentation of UEM (Report 082/2009). Specifically, 30 60-day-old male Wistar rats were studied from the central vivarium of UEM. The animals were maintained under the following conditions: a temperature of 23°C, access to water and balanced

feed (Nuvilab brand – Colombo – PR) *ad libitum* and a photoperiod of 12 h light and 12 h dark. The animals were placed in collective cages (46 X 24 X 20 cm) with five animals each, or in individual cages. One day before the experiment, the animals were anesthetized (0.1 mL/100 g of body weight, muscularly, of a 1:1 mixture of 0.116 g/mL ketamine + 0.02 g/mL xylazine) and a small silicon catheter was implanted into the jugular vein to perform venous infusions and to collect blood samples. Twenty-four hours after the implant, the animals underwent two physiological tests following 12 h of fasting at night.

a) The acute effects of venous infusion on blood glucose.

Two experimental groups were established, the control (n=6) and test groups (n=8). After removing the first blood sample (time-point zero), the animals in the test group received a 5.6 mM solution of transglycosylated that was diluted in an isotonic saline solution (0.9% NaCl). In contrast, the animals in the control group only received the dilution vehicle. Next, new blood samples were collected at 5, 15, 30 and 60 min.

b) Effect of venous infusion on glucose tolerance.

Two experimental groups were established, one control group of animals (n=8) and one test group (n=8). Both groups were subjected to intravenous glucose tolerance testing (GTT), which began at the collection of the first blood sample (time-point zero). Next, the animals from the control group received an intravenous glucose solution (1 g/kg diluted in isotonic saline solution –0.9% NaCl) and the animals in the test group received the same glucose solution that was supplemented with the transglycosylated material (5.6 mM). New blood samples were collected at 5, 15, 30 and 60 min.

In both experiments, an equal volume of isotonic saline solution was infused for each 0.2 mL of collected blood sample to avoid hypovolemia. The blood samples were collected with heparinized syringes that were properly stored in Eppendorf tubes and placed on ice before centrifuging (3,000 rpm for 15 min). The plasma blood glucose level was determined by the oxidase glucose enzymatic method (Kit Gold Analisa)

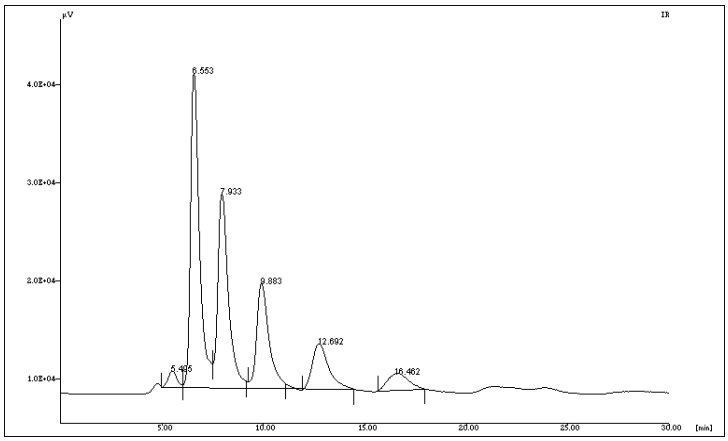
Statistical Analyses: The obtained results are expressed as the means ± the standard error of the mean (SEM). The area under the curve of the blood glucose values was calculated and a t-test was performed for comparison purposes (with a significance of $p<0.05$). The Graphpad Prism 4 statistical software was used.

Sensory analyses

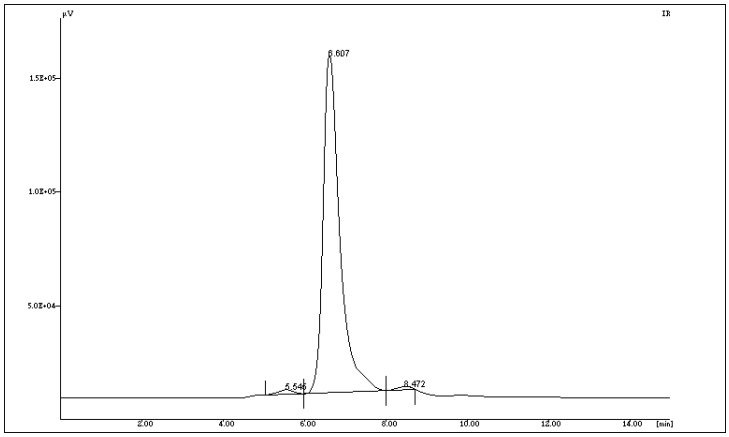
The sensory analyses were performed with 50 untrained tasters by using a nine-point hedonic evaluation scale. The stevioside (starting material) and transglycosylated stevioside materials were evaluated. To prepare the sample (0.1%), 1500 mg of the transglycosylated material were diluted in 1500 mL of mineral water (Crystal brand). Considering that half of the mass of the transglycosylated material was composed of maltodextrin, 750 mg of the starting material (stevioside) were supplemented with 750 mg of maltodextrin before mixing and diluting in 1500 mL of mineral water (Crystal brand). The samples were coded before pouring 10 mL in 50-mL disposable cups for the tasters in individual cabins.

4. RESULTS AND DISCUSSION

Figure 2 (A and B) shows the chromatograms obtained by HPLC/IR for stevioside (starting material) and the stevioside that was enzymatically treated for 24 h. The enzymatically treated stevioside showed a 58% reduction in the area under the curve relative to the starting material. In addition, four additional signals were detected. In the HPLC/UV, similar chromatographic profiles were observed, which demonstrated that the additional peaks most likely correspond to the transglycosylated products from stevioside. These peaks highlighted the presence of stevioside or maltodextrin hydrolysis products.



2A – Chromatogram of the stevioside-rich extract



2B – Chromatogram of the transglycosylated material

The LC/MS analyses (Figures 3 and 4) were used to identify the presence of stevioside ($[M+Na]^+$ = 827) and to determine the molecular ions in the enzymatically treated stevioside, which corresponded to stevioside with one, two or three glucose molecules (m=M/Z 989, 1151 and 1313, respectively). This finding confirmed that the CGTase enzyme promoted the transglycosylation of stevioside.

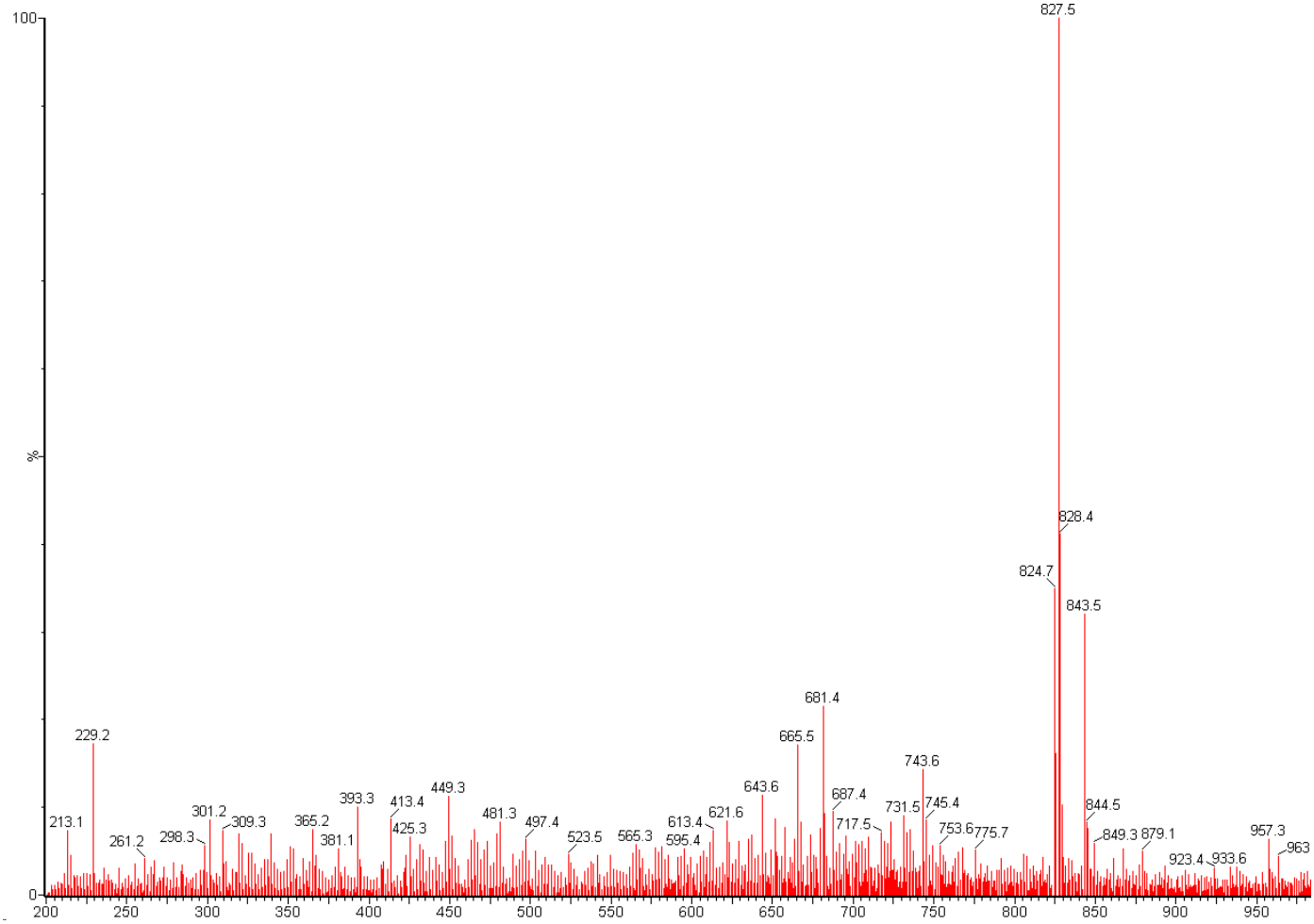


Figure 3: Stevioside analyzed by LC/MS in positive ion mode

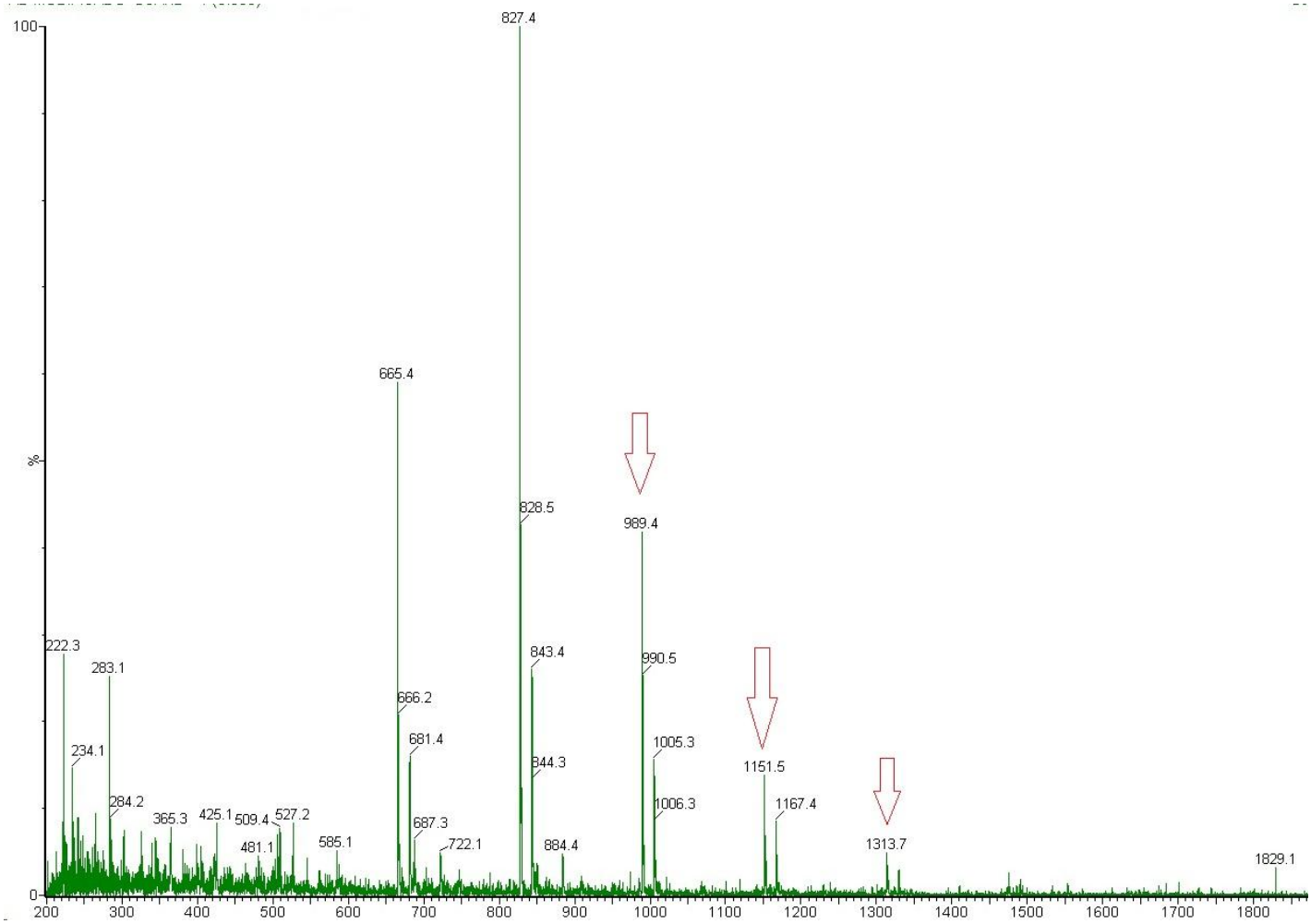


Figure 4: Transglycosylated stevioside analyzed by LC/MS in positive ion mode

The development of new sweeteners can benefit the population, especially individuals with metabolic disorders, such as *diabetes mellitus*, obesity and metabolic syndrome. Such disorders demand strict blood glucose control by the individuals who are affected by them due to the triggered physiological alterations that mainly result from hyperglycemia (Krishnamurti and Steffes, 2001; the diabetes control group, 1993; and UKPDS, 1998). Therefore, experimental animal studies were conducted to evaluate the possible effects of the stevioside transglycosylation products on blood glucose and glucose tolerance in the experimental animals.

Figure 5 shows the venous infusion of transglycosylated materials at a concentration of 5.6 mM and demonstrates that it is possible to observe that no significant effect occurred in the plasma glucose concentrations of normal rats. The blood samples were collected over an interval of 60 min, and no significant differences were observed in the evaluated time points. The area under the curve, which was calculated from the blood glucose values, did not differ (AUC = 228.60±41.71) from the control group (AUC = 223.63±59.39). Thus, no significant differences were observed between the two groups for any of the evaluated time points ($p > 0.05$, t-test).

During the glucose tolerance test, the increase in the blood glucose values that was observed at 5 min was similar in both experimental groups (as demonstrated by Figure 6). However, the blood glucose values decreased in the following time-points. Thus, the areas under the curve did not differ significantly between the groups of animals (AUC = 2043.50 ± 188.09 for the control group and AUC = 2357.50 ± 148.69). Consequently, when transglycosylated material was infused with glucose, the former did not interfere with the removal of blood glucose. Thus, we concluded that the transglycosylated material does not have an effect on glucose tolerance.

These results are important because they indicate that the product is not harmful regarding blood glucose levels. This characteristic is important because it is a sweetener that may be used by diabetic or obese individuals.

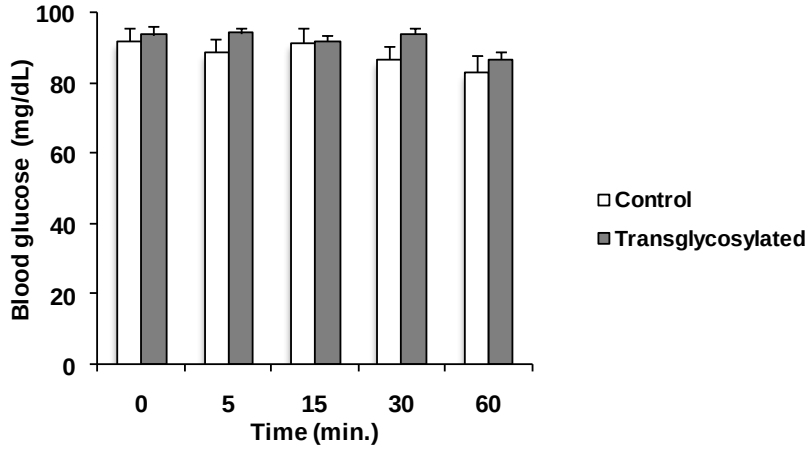


Figure 5. Effect of venous transglycosylated stevioside (5 mM) infusion on blood glucose levels of normal rats (n=8). The control group (n=6) received only the vehicle (isotonic saline solution). The values express the means ± SEM of the plasma glucose values before the infusion (time-point 0) and at the time-points following infusion.

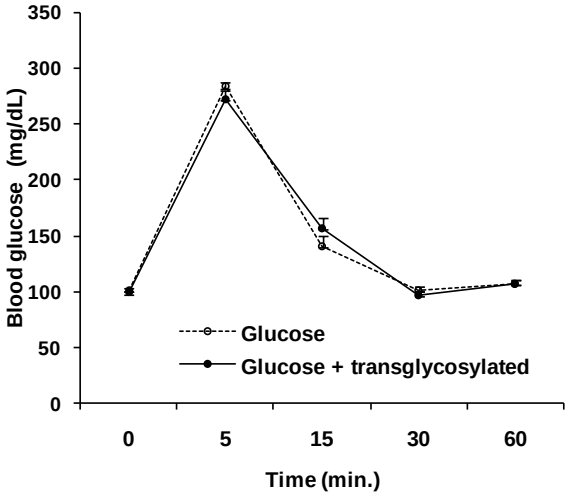


Figure 6. The effects of transglycosylated stevioside (5.6 mM) in the intravenous glucose tolerance test in normal rats. The control group (dashed line) received glucose intravenously (5.6 mM), while the test group (solid line) received glucose (5.6 mM) ± transglycosylated stevioside (5.6 mM) intravenously. The values express the means ± SEM of the values for plasma glucose before the infusion (time-point 0) and at the time-points following infusion.

In the sensory analysis, the tasters evaluated the stevioside (starting material) samples and the transglycosylated material samples for their sweet taste and bitter aftertaste attributes. The mean sweet tastes that were attributed to stevioside and the transglycosylated material were 5.26 and 7.48, respectively. By applying an ANOVA test with 5% significance, no significant differences were observed between samples. For the bitter aftertaste, the mean values for stevioside and the transglycosylated material were 4.52 and 6.92, respectively. When applying an ANOVA test with a significance of 5%, no significant differences were observed between the samples. This result favors the transglycosylated material because a significant improvement in the bitter aftertaste was observed despite the fact that untrained tasters were used. The same sensory analysis also assessed the preferred sample between the

two. In this case, the transglycosylated material was preferred by 86% of the testers relative to 14% for the stevioside.

5. CONCLUSIONS

The results demonstrated the viability using the Toruzyme® commercial enzyme for the transglycosylation of stevioside. The chromatographic analysis confirmed the emergence of new substances in the transglycosylation mixture, specifically stevioside with included glucose molecules. The percentage of the stevioside that was converted in the transglycosylation mixture reached 70.7%. The *in vivo* tests showed the use of the obtained products by people with disorders such as diabetes and obesity is viable. The transglycosylated product obtained excellent sensory acceptance when compared to the traditional stevia sweetener and has potential for industrial production and use as a low-calorie sweetener.

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