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A potential synbiotic product improves the lipid profile of diabetic rats

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Abstract

Background: Previous studies showed that intake of yacon or some lactic acid bacteria was able to inhibit the development of diabetes mellitus, by reducing glucose and associated symptoms, for example, the lipid profile.

Objective: The purpose of this study was to assess the consumption influence of a potential symbiotic product of soybean and yacon extract and fermented *Enterococcus faecium* CRL 183 and *Lactobacillus helveticus* ssp *jugurti* 416 in reducing blood glucose and lipid levels in an animal model.

Methods: Diabetes mellitus was chemically induced by intraperitoneal administration of streptozotocin (50 mg/kg body weight). The rats were divided into four groups (n=10): GI – non-diabetic animals that received only a standard chow diet (negative control), GII – diabetic animals that received only chow diet (positive control), GIII – diabetic animals that received the chow diet + 1 mL/kg body weight/day of soybean and yacon unfermented product, GIV – diabetic rats that received the chow diet + 1 mL/kg body weight/day of soybean and yacon fermented product. There was a seven-week treatment period and the following parameters were evaluated: animal body weight, food and water intake, blood glucose, enzyme activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), triglycerides levels, total cholesterol, HDL-C, non-HDL-C. Cell viability of the fermented product was checked weekly for a seven-week period.

Results: The product average viable population was 10^8-10^9 CFU/mL, by ensuring both the rods and cocci regular intake. No difference was observed between the water and feed intake and body weight of groups that received unfermented and fermented products and the untreated diabetic group. The same was observed for the blood glucose and AST and ALT activities, while some improvement was observed for a lipid profile, represented by reduction of triglycerides level by 15.07% and 33.50% in groups III and IV, respectively, and an increase of 23.70% in HDL-C level for group IV.

Conclusion: The results showed that the ingestion of a potential symbiotic product was neither able to promote improvement in some of the disease symptoms, nor reduce blood glucose. However, a positive effect on triglycerides levels and HDL-cholesterol was observed in the groups that received the unfermented product containing yacon extract and the fermented product with *Enterococcus faecium* CRL 183, as well as *Lactobacillus helveticus* ssp *jugurti* 416 and yacon extract (symbiotic product).

Keywords: Fermented soy product, Probiotics, Prebiotics, Diabetes mellitus, Blood glucose, Lipid profile

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Background

Diabetes mellitus is among the tenth leading causes of death in western countries and, despite progress in its clinical management, it is not yet possible to control its lethal consequences. This disease is a chronic disorder that affects the carbohydrates metabolism, fats and proteins. The characteristic of diabetes mellitus is hyperglycemia, which reflects a deterioration in the use of glucose (and hence carbohydrates in general) due to defective insulin secretion or deficient response to it [1,2]. Diabetes mellitus comprises a number of common symptoms, such as excessive thirst and hunger, weakness, weight loss and rising blood glucose, resulting in excretion of glucose in the urine [3-7]. According to Mori et al. [8], diabetes mellitus is capable of reproducing the hepatic toxic characteristics, increasing the AST and ALT levels in serum blood, therefore, being useful as hepatic biomarkers.

The atherosclerosis risk is twice to three times greater in diabetics than in non-diabetics. The absence of insulin promotes hydrolysis of stored triglycerides in the adipocytes and their presence in the circulation, and the leading to a reduction in serum levels of HDL-C [9-11].

The probiotic foods are those that contain microorganisms that modulate the intestinal microbiota and aid the functioning of the gastrointestinal tract and thus may prevent the disease, while prebiotics are food containing substances which are resistant to enzymatic breakdown, which stimulate the proliferation or activity of certain bacteria in the intestinal microbiota, acting as a selective substrate in the colon. Foods that have both probiotics and prebiotics are called synbiotics [12].

Within this context, fermented foods containing probiotics and prebiotics can be important diet components, due to their nutritional characteristics and ability to reduce the risk of chronic diseases [13-17].

In recent decades, the yacon (*Smallanthus sonchifolius*), a natural plant in the Andean region, has aroused the interest of researchers as it provides bioactive compounds considered important for human health [18]. In Bolivia, it is the potato - like yacon roots are commonly consumed by people suffering from diabetes [19]. In studies of the fructooligosaccharides and inulin found in yacon, lipid-lowering effects have been observed [20].

It has also been reported that some lactic acid bacteria affect the progression of diabetes mellitus [21-25]. These studies show that ingestion of determined lactic acid bacteria prevents or delays the disease onset in various experimental models of diabetes, induced by a chemical or by diet, or genetically modified animals (dB/dB). However, studies relating the use of lactic acid bacteria to the development of diabetes are scarce [26]. A variety of *in vitro* experiments and *in vivo* trials have provided experimental evidence to support the probiotic

roles in lowering serum cholesterol and improving lipid profiles [27].

Considering the probable hypoglycemic effect of yacon, the soybean health beneficial effects and the probiotic lactic acid bacteria [28-32], it seemed timely and of great interest to assess the consumption influence of a potential synbiotic product of soybean and yacon extract and fermented with *E. faecium* CRL 183 and *L. helveticus* ssp. *jugurti* 416 in reducing blood glucose and lipid levels in a streptozotocin-induced diabetes mellitus in rats.

Materials and methods

Animals and experimental protocol

Male wistar rats (n=40), aged 5 weeks (weight 130-170 g), were obtained from the Central Animal Facility of the Campus of Botucatu -UNESP, SP, Brazil. During the 59-day experiment, the animals were kept under controlled temperature (22°C ± 2°C), with a light–dark cycle of 12:12 h, with free access to standard chow and water. The product under study was administered to the animals for seven weeks.

Rats were fed a standard chow diet (Purina®-SP, Brazil) for a week, to acclimatize them, and then they were randomly allocated to four experimental groups (n=10): GI - non-diabetic animals that received only the chow diet (negative control); GII - diabetic animals that received only chow diet (positive control); GIII – diabetic animals that received chow diet + 1 mL/kg body weight/day of soybean and yacon extract unfermented product; GIV – diabetic rats that received chow diet + 1 mL/kg body weight/day of soybean and yacon extract fermented product.

The fermented product, based on aqueous extracts of soybean and yacon, was processed at the Development and Production Unit for Soybean Derivatives (UNISOJA), Food and Nutrition Department of the School of Pharmaceutical Sciences, UNESP in Araraquara, SP, Brazil, by methods described by Pauly-Silveira [33], but all the sugar was replaced by sucralose, because of the diabetic groups condition who consumed the product.

The Foscarin soybean variety (Ribeirão Preto, SP, Brazil) was used in the soy milk processing (raw material of unfermented and fermented products). The hulled grains were submitted to a heat treatment (95°C/7 min) and led to a continuous extracting machine (Perfecta®, Curitiba, PR, Brazil) to obtain the soy milk. The used yacon was of yellow variety and was purchased in local marketing. Immediately after yacon cutting, the pieces were blanched in hot water (97°C) and citric acid was added, for a 15-min period. Finally, the yacon was ground and filtered to obtain the aqueous extract [33]. The bacterial inocula consisted of 3.0% (v/v) of a 1:1 mixture of *Enterococcus faecium* CRL 183 (probiotic microorganism from CERELA - Argentina) and *Lactobacillus helveticus*

ssp. *jugurti* 416 (adjuvant fermentation from Institute of Food Technology - ITAL - Campinas, SP, Brazil). The aqueous extracts mixed at soybean rates: yacon of 60.00% to 25.86% [33]. The fermentation was conducted at temperatures of 37°C until the pH of 4.5 was reached. The unfermented and fermented products showed the following proximal composition (%): moisture 82.85; total solids 17.15; protein 3.97; fat 2.49; ash 0.51; carbohydrates 10.19.

The unfermented product was prepared by chemical acidification of the synbiotic product base mixture (without bacterial culture) with food-grade lactic acid (Purac, Sao Paulo, Brazil), to reach the same pH as the fermented product (4.4 - 4.6).

After the period of adaptation, the rats were fasted for 12 h. Afterwards, the rat group II; then, the groups III and IV by injections (intraperitoneal) of 50 mg per kg body weight of streptozotocin (STZ) (Sigma Aldrich®, St Louis, MO, USA) [34,35] dissolved in 10 mM citrate buffer (pH 4·5), while animals of group I received a similar buffer injection. Three days later, the blood glucose level of STZ-treated rats had risen to 500 mg/dL, while group I remained around 120 mg/dL.

The unfermented and fermented administration products started after confirmation of diabetes induction. The products were administrated to the animals by gavage of 1 mL/kg body weight once every day, to ensure a minimal intake of 10^7 CFU/day of *E.faecium* and of *L.helveticus* ssp *jugurti*, in the fermented products for a seven-week period. The fermented product contained 10^8- 10^9 CFU/mL of each microorganism.

The experimental design received approval from the Research Ethics Committee of the School of Pharmaceutical Sciences, UNESP in Araraquara, SP, Brazil (protocol CEP/FCF no. 21/2010)

Measurement of total fructans
The total fructan content was measured in triplicate by a spectrophotometric method and was carried out in yacon extract, in the unfermented and potential synbiotic products (Amersham Biosciences® model Ultrospec 3100 pro, USA) using the Megazyme Fructan HK Assay kit (AOAC Method 999.03 and AACC Method 32.32; Megazyme International Ireland Ltd., Wicklow, Ireland) [36].

Body weight, food and water intake
The body weight of each animal was recorded weekly throughout the experimental period.

The feed and water intakes of each animal were determined by weight difference between chow offered and orts and the difference between the amounts of water offered from remaining bottles, respectively, and recorded daily throughout the experimental period.

Sample collection
Blood samples were collected from a small longitudinal incision made at the animal tail end [37].

Glycemia
The level of glucose in the plasma was assessed at the beginning of the experiment, three days after induction of diabetes, to verify the diabetic state, 14 days after proof of the diabetic state and at the end of the experiment, after the final administration of the products, using Labtest Diagnostic® Kit SA (Lagoa Santa, MG, Brazil) [38,39].

Liver enzymes (aspartate aminotransferase and alanine aminotransferase)
The enzymatic activity of the transaminases AST and ALT was determined in the plasma by Labtest Diagnostic® Kit SA (Lagoa Santa, MG, Brazil), at the same time as the glucose analysis [38,39].

Serum lipids
Lipids were determined in the plasma, by using Labtest Diagnostic® Kit SA (Lagoa Santa, MG, Brazil) [38,39]. nHDL-C was calculated by the equation: nHDL-C=Total Cholesterol – HDL-C [40].

Euthanasia of animals
All experimental animals were decapitated by guillotine at the end of the experiment (after seven weeks of treatment).

Statistical analysis
Results are presented as mean ± standard deviation. One-way ANOVA was used to determine a significant difference between groups ($p < 0.05$) and Tukey's test to perform multiple comparisons between means.

Results
Content of total fructans
The total concentration of fructans found in the aqueous yacon extract was 7.96 g/100 g; 4.32 g/100 g in the unfermented product and 4.30 g/100 g in the symbiotic product. The concentrations of unfermented and synbiotic product did not show statistical difference ($p < 0.05$), suggesting that the microorganisms used in fermentation process were not able to modify the total fructans level.

Body weight, feed and water intake
Table 1 shows the average consumption of water and feed as well as change in body weight throughout the experimental period; the evident increase in hunger and thirst in groups II, III and IV confirmed the induction of diabetes.

It is observed that for the three variables presented in Table 1, the diabetic control group (II) did not differ statistically from the other diabetic groups (III and IV). The

Table 1 Average daily intakes of water and feed and body weight of animals in non-diabetic and diabetic groups during the trial period

Groups Parameters	Group I	Group II	Group III	Group IV
Water consumption (mL)	41.26[b] ± 0.81	173.45[a] ± 10.63	192.16[a] ± 9.20	183.46[a] ± 9.16
Feed consumption (g)	25.47[b] ± 0.26	38.89[a] ± 1.20	39.56[a] ± 1.41	38.78[a] ± 1.31
Body weight (g)	306.47[b] ± 24.33	242.41[a] ± 8.10	245.49[a] ± 8.23	235.29[a] ± 6.93

Values are expressed as mean ± standard error (n=10). Means values for groups with same lowercase letter on the same line do not differ significantly at p ≤ 0.05. Group I = non-diabetic animals that received only chow diet (negative control), Group II = diabetic animals that received only chow diet (positive control), Group III = diabetic animals that received chow diet + 1 mL/kg bw/day of soybean and yacon unfermented product, Group IV = diabetic animals that received chow diet + 1 mL/kg bw/day of soybean and yacon fermented product.

consumption of water and feed was greater for all diabetic rats and their weight was lower than for non-diabetic rats.

Glycemia
In Table 2 there are the results about glucose levels.

Liver enzymes
The AST and ALT serum levels were presented in Table 3.

Regarding the enzyme alanine aminotransferase (ALT), there were significant differences among group I and the others, starting from time T2. Note also that all diabetic groups showed a significant increase in ALT activity after seven weeks of treatment, but noting that, once again, groups II, III and IV did not differ significantly from each other.

Serum lipids
Table 4 shows the values of the lipid profile of the four groups throughout the experimental period.

It is observed that, at the end of the experiment, triglycerides levels were lower in the groups receiving treatment (III and IV) than in the positive control (group II), although these levels are higher than that observed in group I.

As for total cholesterol, it is clear that at the end of the experiment (T3), groups II, III and IV did not differ, while they were significantly different from group I.

Regarding HDL-C at the end of the experiment (T3), group IV, which consumed the potential symbiotic product, differed from all the other groups, with the highest value for this parameter. On the other hand, there was no statistical difference in non HDL-C among any of the groups during the experiment.

Discussion
The fructans content, determined in the unfermented and symbiotic product, was found to be compatible with recommended daily intake of prebiotics (4-5 g/day) to stimulate the growth of *Bifidobacterium* [41], while doses of 3 to 10 g/day promote reduction of blood pressure, have a beneficial effect on lipid metabolism, improve gastrointestinal health [42] and provide hypoglycemic effect [43]. Although a high content of fructans in the unfermented and symbiotic products was found, a hypoglycemic effect was not observed, but it may have been one of the factors that contributed to reducing the triglycerides in groups III and IV. In addition, some studies suggest that caffeic acid, chlorogenic acid (3-caffeoylquinic acid) and very probably other caffeic acid derivatives, such as 3,4-dicaffeoylquinic, 3,5-dicaffeoylquinic and 4,5-dicaffeoylquinic acids, are also the

Table 2 Plasma glucose levels of groups of non-diabetic and diabetic animals during the trial period

Groups Time Glucose	Group I	Group II	Group III	Group IV
T0	117.20[B,a] ± 2.22	119.10[C,a] ± 2.60	121.00[C,a] ± 1.78	125.40[C,a] ± 2.36
T1	129.20[AB,b] ± 3.71	503.14[B,a] ± 25.17	504.67[B,a] ± 22.68	534.50[B,a] ± 21.02
T2	103.36[C,b] ± 3.97	633.00[A,a] ± 29.43	657.57[A,a] ± 11.31	652.67[A,a] ± 9.60
T3	134.46[A,b] ± 2.41	538.60[B,a] ± 14.05	551.29[B,a] ± 12.97	554.33[B,a] ± 12.84

Values are expressed in mg/dL (n=10, mean ± standard error). Means values for groups with lowercase letters on the same line do not differ significantly at p ≤ 0.05; comparing times, means with same capital letters in the same column do not differ significantly p ≤ 0.05. Group I = non-diabetic animals that received only chow diet (negative control), Group II = diabetic animals that received only chow diet (positive control), Group III = diabetic animals that received chow diet + 1 mL/kg bw/day of soybean and yacon unfermented product, Group IV = diabetic animals that received chow diet + 1 mL/kg bw/day of soybean and yacon unfermented product. T0 = initial blood collection, T1 = blood collection after a week to confirm the induction of diabetes mellitus, T2 = blood collection four weeks to detect any reversal of the diabetic condition, T3 = blood collection when sacrificed at end of experiment (7 weeks).

Table 3 Concentrations of liver enzymes in non-diabetic and diabetic groups during the trial period

Group Parameters/ Time	Group I	Group II	Group III	Group IV
AST				
T0	164.18[B.a] ± 7.48	168.78[B.a] ± 9.55	163.45[A.a] ± 7.72	159.00[B.a] ± 10.22
T1	156.50[B.a] ± 9.33	159.75[B.a] ± 15.15	180.10[A.a] ± 11.90	175.7[AB.a] ± 12.64
T2	208.09[A.a] ± 8.24	283.90[A.a] ± 35.68	219.36[A.a] ± 14.85	254.20[A.a] ± 29.05
T3	150.45[B.a] ± 9.20	166.56[B.a] ± 29.97	171.64[A.a] ± 25.03	164.25[B.a] ± 16.97
ALT				
T0	74.60[AB.a] ± 3.49	78.25[B.a] ± 2.59	73.18[B.a] ± 2.98	67.50[C.a] ± 1.57
T1	63.10[C.a] ± 2.25	67.5[B.a] ± 1.88	61.46[B.a] ± 1.87	59.25[C.a] ± 2.43
T2	68.90[B.b] ± 1.75	126.80[A.a] ± 18.05	151.63[A.a] ± 10.80	136.80[B.a] ± 12.38
T3	83.80[A.b] ± 2.61	150.00[A.a] ± 9.25	163.00[A.a] ± 12.85	163.83[A.a] ± 5.50

Values are expressed in U/L (n=10, mean ± standard error). Comparisons between groups: mean values with the same lowercase letter on the same line do not differ significantly at $p \leq 0.05$, and times: equal means with capital letters in the same column do not differ significantly $p \leq 0.05$. Group I = non-diabetic animals that received only chow diet (negative control), Group II = diabetic animals that received only chow diet (positive control), Group III = diabetic animals that received chow diet + 1 mL/kg bw/day of soybean and yacon unfermented product, Group IV = diabetic animals that received chow diet + 1 mL/kg bw/day of soybean and yacon unfermented product. T0 = initial blood collection, T1 = blood collection after a week to confirm the induction of diabetes mellitus, T2 = blood collection after four weeks to detected any reversal of the diabetic state, T3 = blood collection when sacrificed at end of experiment (seven weeks).

active principles related to the hypoglycemic effect of yacon leaves [44,45].

The highest feed intake observed in the diabetic groups can be explained by the absence of circulating insulin, which causes a deficiency in glucose transport, leading to a deficiency of energy in the cells, resulting in increased feeding to compensate for that lack of energy [46].

In addition, it can be explained by physiological processes related to the pathology, such as hyperglycemia,

Table 4 Lipid profile in non-diabetic and diabetic groups during the trial period

Groups Parameters/ Time	Group I	Group II	Group III	Group IV
Triglycerides				
T0	135.10[B.a] ± 12.62	173.73[B.a] ± 11.46	156.64[B.a] ± 12.51	175.36[C.a] ± 13.37
T2	188.82[A.b] ± 12.90	425.33[AB.a] ± 84.89	396.18[A.a] ± 49.70	348.50[AB.a] ± 31.14
T3	149.55[AB.c] ± 9.95	628.67[A.a] ± 32.05	503.25[A.b] ± 25.79	419.00[A.b] ± 35.39
Total cholesterol				
T0	101.37[A.a] ± 4.66	111.73 [A.a] ± 3.33	99.64[A.a] ± 2.81	98.9[A.a] ± 2.71
T2	80.78[B.b] ± 3.30	100.79[A.a] ± 4.97	102.46[A.a] ± 3.73	100.25[A.a] ± 4.07
T3	69.82[B.b] ± 5.52	96.78[A.a] ± 5.61	97.64[A.a] ± 2.96	90.33[A.a] ± 4.84
HDL-C				
T0	45.27[A.a] ± 1.92	55.82[A.a] ± 3.64	50.09[A.a] ± 1.66	52.82[A.a] ± 3.25
T2	43.82[A.a] ± 2.59	52.40 [A.a] ± 2.20	48.64[A.a] ± 1.50	48.70[A.a] ± 2.25
T3	34.37[B.c] ± 2.13	52.44[A.b] ± 1.70	52.27[A.b] ± 1.61	65.00[A.a] ± 6.36
nHDL-C				
T0	39.56[B.a] ± 2.35	48.57[A.a] ± 2.81	48.63[A.a] ± 2.15	45.50[A.a] ± 3.01
T2	35.46[B.a] ± 4.19	48.13[A.a] ± 3.93	45.37[A.a] ± 2.66	39.40[A.a] ± 2.16
T3	56.09[A.a] ± 3.60	55.91[A.a] ± 3.88	49.55[A.a] ± 2.92	46.09 [A.a] ± 3.19

Values are expressed in mg/dL (n=10, mean ± standard error). Means values for groups with lowercase letters on the same line do not differ significantly at $p \leq 0.05$; comparing times, means with same capital letters in the same column do not differ significantly at $p \leq 0.05$. Group I = non-diabetic animals that received only chow diet (negative control), Group II = diabetic animals that received only chow diet (positive control), Group III = diabetic animals that received chow diet + 1 mL/kg bw/day of soybean and yacon unfermented product, Group IV = diabetic animals that received chow diet + 1 mL/kg bw/day of soybean and yacon unfermented product. T0 = initial blood collection, T2 = blood collection after four weeks to detect any reversal of the diabetic condition, T3 = blood collection when sacrificed at end of experiment (seven weeks).

glycosuria, and polyuria, while the weight loss is due to the catabolic processes involved in diabetes mellitus [47].

While in the non-diabetic group (group 1), the animals appeared, throughout the experiment, in good general condition, with normal appetite, progressive weight gain and maintenance of water intake, food intake and diuresis, the diabetic animals had a strong odor of urine and functional changes, such as polyuria and polydipsia. However, there were no significant changes of the coat or apathy, as described by Passos and Park [48].

In the present study, the increased serum glucose levels in groups II, III and IV confirmed that the STZ was effective in inducing diabetes mellitus, values of around 120 mg/dL rising to nearly 500 mg/dL, a level correlated with severe experimental diabetes [49].

In the blood tests used to check for induction reversal (T2), the highest blood glucose levels were recorded, thus confirming the absence of reversion. The rate of induction was 100%, since long-term diabetes mellitus was found in all animals, as observed by Rakieten et al. [50], in rats and dogs, at the same doses of 50 mg STZ per kg body weight.

At T0, there was no statistical difference in glycemic level among the groups. After induction and until the end of the experiment, only group I (non-diabetic) maintained low values, by significantly differing from the other groups, which all had become diabetic.

Although a reduction in blood glucose occurred at the end of the experiment, it cannot be suggested that, thus revealed a tendency for diabetes to stabilize, owing to the presence of yacon and/or probiotic bacteria, since the animals in group II received only water and feed and showed the same reduction. Studies by Maffezzoli [51] showed results similar to those in this study.

The organism itself tends to normalize high blood glucose levels by three main routes: stimulating glucose uptake by peripheral tissues (muscle and adipose tissue); altering the insulin metabolism (by reducing the degradation of insulin in the liver or stimulating insulin secretion) and, finally, by inhibiting the reabsorption of glucose by the kidneys, resulting in the elimination of glucose in the urine [52].

The glycemic control observed in animal studies and early studies in humans has indicated that ingested fructooligosaccharides probably act to stimulate glucose utilization in peripheral tissues (muscle and adipose tissue) [52].

The enzymes analyzed in this study are markers of liver injury; the observed increase in AST and ALT activity indicates an aggressive hepatocyte injury [53] with regard to these enzymes, it was noted that there was no significant difference among the three diabetic groups, indicating again that the presence of yacon extract did not improve the condition of the liver of animals in groups III and IV.

A study by Baroni et al. [54] evaluated the activity of AST and ALT in the plasma of diabetic and non-diabetic animals that received 10% hydroethanolic extract of yacon for 14 days. These researchers observed that the activity of these enzymes increased in diabetic rats but that, on administration of the extract, the enzyme activities of diabetic rats were close to those of control animals. One possible explanation is that the administration of the yacon extract may have decreased the hepatic lesions caused by the disease. However, these results were not confirmed in our study.

Regarding the lipid profile, it was observed that at the end of the experiment, triglycerides levels were lower in groups III and IV than in group II, although all these levels were higher than that observed in group I, indicating that both the unfermented and fermented products may have reduced this serum lipid in STZ-induced animals. Numerically, group IV had the lowest triglycerides, which may be related to the aqueous yacon extract [55] and the probiotic one [56] used in the synbiotic product.

Whereas diabetes results in increased lipolysis in adipose tissue, leading to higher blood levels of fatty acids, there is also a greater production of ketone bodies by the liver. However, the excess fatty acid captured by the liver is not fully oxidized to ketone bodies by ketogenesis. Thus, these excess fatty acids are directed to the synthesis of triglycerides, which is converted into VLDL. As VLDLs in excess are not be fully metabolized by lipoprotein lipase, a state of a hypertriglyceridemia would occur [57].

As for total cholesterol, note that at the end of the experiment groups II, III and IV did not differ, although they were significantly different from group I. However, group IV again showed, numerically, the lowest value for this parameter, although not statistically different from the others; that may be an indicative of a tendency, in agreement with the results observed by Rossi et al. [58], which showed that E. faecium CRL 183 was able to reduce total cholesterol levels by 18.4% in hyperlipidemic rabbits.

A variety of past in vitro experiments and in vivo trials have provided evidence to support the roles of probiotics in lowering serum cholesterol and improving lipid profiles.

Several mechanisms of cholesterol reduction by probiotics via control of cholesterol metabolism have been proposed. One such mechanism is the removal of cholesterol by assimilation. The assimilation of cholesterol by probiotics in the small intestine could reduce serum cholesterol by reducing the absorption of cholesterol in the intestines [59]. Probiotics must be viable and growing, in order to be able to remove or assimilate cholesterol [60]. Other researchers have suggested that the incorporation of cholesterol into cell membranes could be another mechanism used to reduce cholesterol in media, or that it involves the ability of certain probiotics to deconjugate bile acids enzymatically [61].

Regarding HDL-C at the end of the experiment (T3), group IV (symbiotic) differed from the other groups, with the highest value for this parameter, which may be due to the presence of *E. faecium* CRL 183. This ability of *E. faecium* CRL 183 to promotion increase in HDL-C has been observed in a study by Rossi et al. [58], where the authors showed an increase of this fraction by 17.8%.

In comparison to the results of Khamisy [62], who administered a suspension of *Bifidobacterium* and *Lactobacillus acidophilus*, separately or in combination to diabetic albino rats, the presented results were more satisfactory in terms of the increase in HDL-C in group IV, which received the symbiotic product; this is an important condition in reducing the risk of onset of coronary heart disease in diabetics.

In another study conducted by Manzoni et al. [17], concerning the beneficial effects on rat serum lipid effects of soy yogurt fermented with *E. faecium* CRL 183, only HDL levels were changed positively, by showing a 46% increase.

Research indicates that the hypocholesterolemic action of probiotics may be enhanced by the use of a prebiotic [63]. The effects of probiotic combinations and certain prebiotics i.e. symbiotic on blood lipids were investigated by Kiebling et al. [64] and Greany et al. [65]. One study reported that the ingestion of yogurt fermented with *L. acidophilus* 145 and *B. longum* 913 plus 1% oligofructose [64] raised the level of HDL-C, while another, which tested the combination *L. acidophilus* DDS-1, *B. longum* UABL-14 and fructooligosaccharides, found no effect [65].

Regarding the fraction nHDL-C, no statistical difference was observed among the 4 groups during the experimental period.

Conclusion

The product based on aqueous extracts of yacon and soybean fermented or not with *Enterococcus faecium* CRL 183 and *Lactobacillus helveticus* ssp *jugurti* 416, showed no positive effect on blood glucose levels, but was effective in reducing triglycerides both unfermented and symbiotic products and increasing HDL-cholesterol (symbiotic product alone).

Abbreviations
AST: Aspartate aminotransferase; ALT: Alanine aminotrasferase; HDL-C: High density lipoprotein cholesterol; n-DHL-C: Non high density lipoprotein cholesterol; VLDL: Very low density lipoprotein; STZ: Streptozotocin.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
MNR: was involved in design, data collection, drafting the manuscript and revising it critically for important intellectual content. NDPS, DCUC, LSC, RCV, RAP: participated in data collection, interpretation of results and drafting the manuscript. EAR: was involved in design, drafting the manuscript and revising it critically for important intellectual content. All authors read and approved the final manuscript.

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