Cashew juice containing prebiotic oligosaccharides

Isabel Moreira da Silva · Maria Cristiane Rabelo ·
Sueli Rodrigues

Revised: 5 February 2012 / Accepted: 14 March 2012 / Published online: 5 April 2012
© Association of Food Scientists & Technologists (India) 2012

Abstract The enzyme dextranase in a medium containing sucrose and an acceptor as substrate synthesizes prebiotics oligosaccharides. The cashew apple juice works as a source of acceptors because it is rich in glucose and fructose (enzyme acceptors). The use of cashew apple juice becomes interesting because it aims at harnessing the peduncle of the cashew that is wasted during the nut processing, which is the product of greater economic expression. The production of dextranase enzyme was done by fermentative process by inoculating the bacterium *Leuconostoc mesenteroides* NRRL B512F into a culture medium containing sucrose as the only carbon source. Thus, the aim of this work was the production of prebiotic oligosaccharides by enzymatic process with addition of the dextranase enzyme to the clarified cashew apple juice. Dextran yield was favored by the combination of low concentrations of sucrose and reducing sugars. The formation of oligosaccharides was favored by increasing the concentration of reducing sugars and by the combination of high concentrations of sucrose and reducing sugars, the highest concentration of oligosaccharides obtained was 104.73 g/L and the qualitative analysis showed that at concentrations of 25 g/L and 75 g/L of sucrose and reducing sugar, respectively, it is possible to obtain oligosaccharides of degree of polymerization up to 12. The juice containing prebiotic oligosaccharide is a potential new functional beverage.

Keywords Dextranase · *Leuconostoc mesenteroides* B512F · Clarified cashew apple juice · Prebiotic oligosaccharides

Introduction

A functional food is similar in appearance to a conventional food, however was modified with ingredients that provide additional health effects. Prebiotic is defined as a food component not digested in stomach and selectively digested by *Bifidobacteria* and *Lactobacillus* in the intestine (Fernando et al. 2011). Thus, prebiotic oligosaccharides beneficially affect the host by selectively stimulating the growth and/or activity of only one or a limited number of bacteria in the colon improving the host health (Madhukumar and Muralikrishna 2011; Gibson and Roberfroid 1995).

Prebiotics oligosaccharides have been used as new functional ingredients of foods showing great potential to improve the quality of many foods including milk drinks and functional symbiotic. For a food ingredient to be classified as a prebiotic is necessary: not undergo hydrolysis and not be absorbed in the upper gastrointestinal tract; be a selective substrate for a limited number of potentially beneficial bacteria to the colon, which are stimulated to grow and develop metabolic activities; be able to promote a healthy intestinal biota and, consequently, induce effects in the lumen that benefit the host (Manosroi et al. 2011; Vergara et al. 2010).

Dextranase catalyzes the formation of dextran when in a medium containing sucrose as sole substrate. However, in medium containing an acceptor (maltose, fructose, glucose, etc.) as substrate, besides sucrose as the second substrate, the enzyme produces prebiotic oligosaccharides (Rodrigues et al. 2006; Rabelo et al. 2006). This reaction is called the acceptor reaction and the ratio sucrose:acceptor affects the dextran and oligosaccharides yield and the degree of polymerization (Rabelo et al. 2006; Rodrigues et al. 2006).

I. M. da Silva · S. Rodrigues (✉)
Centro de Ciências Agrárias, Departamento de Tecnologia de Alimentos – DTA, Universidade Federal do Ceará,
Campus do Pici – Bloco 858,
CEP 60021-970 Fortaleza, CE, Brazil
e-mail: sueli@ufc.br

M. C. Rabelo
Centro de Tecnologia, Departamento de Engenharia Química,
Universidade Federal do Ceará,
Campus do Pici Bloco 709,
CEP 60455-760 Fortaleza, CE, Brazil

The acceptor reaction is due to the addition of glucose units from sucrose breakage to the acceptor molecule (glucose, fructose, maltose, etc.) instead of dextran chain (Rabelo et al. 2006; Rodrigues et al. 2006). As glucose units are incorporated into oligosaccharides chain, the degree of polymerization increases. Degrees of polymerizations up to 10 units of glucose, with glucose being incorporated by enzymatic synthesis through α -1,6 glycosidic bonds are known as prebiotic oligosaccharides. These oligosaccharides, although present relevant technical applications, are considered of difficult synthesis (Heincke et al. 1999; Rabelo et al. 2006; Rodrigues et al. 2006).

Cashew is composed of two parts: the nut and the peduncle, which is underutilized (Silveira et al. 2010). The product of greater economic expression is the nut, which is a typical product for exportation, while the products obtained by processing the peduncle (jams, juice, ice-creams and others) are basically intended to the local market (Chagas et al. 2007). Cashew apple juice is rich in glucose and fructose (Fontes et al. 2009) and thus may act as a source of acceptors. Nowadays, almost all functional foods containing prebiotic oligosaccharides are milk based products such as enriched milks. Furthermore, the use of low cost regional substrates such as cashew juice aimed the rational use of a large available raw material, besides the development of a new functional food. Previous studies have demonstrated the potentiality of cashew apple juice as functional food (Vergara et al. 2010; Rabelo et al. 2009). However, in these studies fermentation process using yeast extract were applied imparting undesirable aroma and flavor to the product. In the present work, the prebiotic oligosaccharide was synthesized direct into the juice, because the enzyme showed remarkable stability (96 h) in cashew apple juice at room temperature (Honorato and Rodrigues 2010).

Materials and methods

Strain activation

A strain of *L. mesenteroides* NRRL B-512F obtained from ARS Culture Bacterial Collection (NRRL Culture collection, United States Department of Agriculture, Peoria, IL, USA) was activated in an optimized synthetic medium composed of sucrose, 50 g/L (food grade); yeast extract, 20 g/L (Himedia); $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.20 g/L; $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$, 0.01 g/L; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g/L; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.02 g/L; NaCl, 0.01 g/L; and K_2HPO_4 (anhydrous), 20 g/L. The initial pH of the culture medium was adjusted to 6.5 with H_3PO_4 and the medium was autoclaved (121 °C/15 min). Activation was carried out in an orbital shaker (Tecnal TE 420) at 30 °C and 150 rpm (Rabelo et al. 2006; Rodrigues et al. 2003) for 12 h. This cell culture was used as inoculums to produce dextransucrase in a fed

batch fermentation process. The inoculums amount was 3 % (v/v) of the culture media volume.

Enzyme preparation

Fermentation was carried out in a 1 L TecBio Fermentor containing 500 mL of the culture medium above described. The process was carried out at 30 °C with pH controlled at 6.5 (± 0.1); mechanical agitation of 150 rpm and aeration of 0.5 L/min. A solution containing NaOH (120 g/L) and sucrose (300 g/L) was fed into the fermentator to control de pH and to avoid total sucrose depletion in the culture medium. The feed flow rate was intermittent and automatically controlled to keep the pH value at 6.5 for 6 h of fermentation (Rabelo et al. 2006). After 6 h of fermentation, the feed flow rate was interrupted and the pH was allowed to drop to 5.2 (optimum pH of enzyme activity and stability) when the process was considered finished. The whole process was carried out in 8 h.

The cells were harvested by centrifugation at 11,600g for 10 min at 4 °C in a Sigma 6 K-15 centrifuge. The enzyme was recovered by precipitation with polyethylene glycol (PEG 1500 50 % v/v). The partially purified enzyme was then diluted in a sodium acetate buffer (20 mM) containing 0.05 g/L of CaCl_2 with pH adjusted to 5.2. The enzyme was stored frozen at -20 °C prior to use.

Enzyme activity assay

Enzyme activity was determined by quantifying the released fructose by the DNS (3,5-dinitrosalicylic acid) method (Miller 1959). The partially purified enzyme prepared as described above (45 mL) was mixed with 455 mL of a 10 % (w/v) sucrose solution in sodium acetate buffer pH 5.2 (20 mM containing 0.05 g/L of CaCl_2) and then incubated for 1 h at 30 °C. Samples of 100 mL were collected every 15 min and mixed with 100 mL of the DNS reagent to stop the reaction. The enzyme activity was calculated by linear regression and expressed in IU/mL (dextransucrase unit/mL). One international unit is defined as the amount of enzyme that releases 1 mmol of fructose per minute under ideal reaction conditions (30 °C and pH 5.2) as described by Rabelo et al. (2009).

Raw material for enzyme synthesis

Cashew apple (*Anacardium occidentale* L.) juice was obtained through mechanical process. The juice, which contains high levels of tannins, was clarified by adding gelatin to remove tannins and suspended solids (Honorato et al. 2007). The pH of the juice was determined by direct measurement in a Marconi PA 200 potentiometer. Reducing sugars were determined by the HPLC as further described.

Enzymatic synthesis of prebiotic oligosaccharides

Syntheses were carried out at optimum synthesis conditions (30 °C and pH 5.2) in a thermostated glass batch reactor containing 10 mL of the clarified cashew apple juice. The juice pH was adjusted to 5.2 with NaOH 1 M. Glucose, fructose and sucrose levels were changed (Table 1) according to a two-level central composite design with three central points. Enzyme activity was 0.5 IU/mL. Reducing sugar concentrations used in the experimental design were obtained from the dilution of clarified cashew apple juice that showed a total concentration of 87.7 g/L. Cashew apple juice does not contain sucrose, thus this carbohydrate was added to the juice to promote the synthesis. The analyzed responses were dextran and oligosaccharides.

Syntheses were performed until total consumption of sucrose, which was calculated according to the definition of enzyme activity and confirmed by high-performance liquid chromatography (HPLC) analysis. Dextran was precipitated by adding 3 vol of 96 % (v/v) ethanol. The supernatant was used to quantify the carbohydrates. The precipitated dextran was diluted in distilled boiling water and assayed as total carbohydrate by phenol-sulfuric acid method (Dubois et al. 1956).

Prebiotics oligosaccharides characterization and quantification

The prebiotics oligosaccharides were characterized according to its degree of polymerization by Thin Layer Chromatography (TLC) on Whatman K6 silica plates, 250-μm thickness (Whatman, Kent, UK). Diluted samples of 5 μL were absorbed onto the plates on a line about 1.5 cm above the lower plate edge. After drying with a hairdryer, the plate was irrigated for two ascents in a solvent mixture composed of acetonitrile:ethyl acetate:1-propanol: H₂O (85:20:50:90). To make sugars visibles, the plates were sprayed to saturation with a solution containing 0.3 % (w/v) of 1-naphthyl

ethylenediamine dihydrochloride in methanol with 3 % (v/v) sulfuric acid. The plates were then heated in an oven at 120 °C until spots were visible (approximately 10 min).

The residual sugars were analyzed by high performance liquid chromatography (HPLC) in a Varian ProStar system equipped with two high-pressure pumps model ProStar 210, refraction index detector model ProStar 355 RI and column oven (Timberline). Separation was achieved using a Aminex® (300 mm×7.8 mm) column at 85 °C. Ultra pure water at 0.8 mL/min was used as eluent and the detector temperature was 35 °C. All samples were analyzed in triplicate. The software ProStar WS 5.5 was used to acquire and handle the data. Oligosaccharide concentration was calculated by mass balance (Eqs. 1 to 6). Yields were calculated by Eqs. 7 and 8. Dextran concentration was assayed by colorimetric method as earlier described.

$$F_i(\text{g/L}) = \frac{TRS + Suc}{2} \quad (1)$$

$$F_{Cons}(\text{g/L}) = F_i - RF \quad (2)$$

$$G_i(\text{g/L}) = \frac{TRS + Suc}{2} \quad (3)$$

$$G_{Cons}(\text{g/L}) = \left(\frac{TRS}{2} + G_i \right) - RG \quad (4)$$

$$Oligo(\text{g/L}) = F_{Cons} + G_{Cons} \quad (5)$$

$$TS(\text{g/L}) = Suc + TRS \quad (6)$$

Table 1 Experimental design and results: dextran, dextran yield, oligosaccharides and oligosaccharide yield synthesized in cashew apple juice by dex-transucrase enzyme

Run	Sucrose (g/L)	Reducing sugar (g/L)	Dextran (g/L)	Y _{DXT} (%)	Oligosaccharides (g/L)	Y _{OLIG} (%)
1	25.00	25.00	7.21±0.31	14.42	27.04±0.56	54.08
2	25.00	75.00	8.17±0.29	8.17	53.49±0.67	53.49
3	75.00	25.00	7.44±0.12	7.44	71.20±0.98	71.20
4	75.00	75.00	4.85±0.12	3.23	104.73±0.78	69.82
5	14.64	50.00	6.71±0.18	10.38	38.55±0.56	59.64
6	85.35	50.00	9.63±0.06	7.11	62.94±0.59	46.50
7	50.00	14.65	10.64±0.00	16.46	41.65±0.77	64.42
8	50.00	85.35	11.21±0.01	8.28	77.96±0.32	57.60
9	50.00	50.00	13.60±0.10	13.60	63.84±0.23	63.84
10	50.00	50.00	13.58±0.06	13.58	59.24±0.45	59.24
11	50.00	50.00	13.44±0.18	13.44	65.18±0.87	65.18

$$Y_{Olig}(\%) = \frac{Olig(g/L)}{TS(g/L)} \times 100 \quad (7)$$

$$Y_{DXT}(\%) = \frac{DXT(g/L)}{TS(g/L)} \times 100 \quad (8)$$

Where

DXT	dextran (g/L)
F _{Cons}	consumed fructose (g/L)
F _i	initial fructose concentration (g/L)
G _{Cons}	consumed glucose (g/L)
G _i	initial glucose (g/L)
Oligo	oligosaccharides (g/L)
RF	residual fructose (g/L)
RG	residual glucose (g/L)
Suc	initial sucrose concentration (g/L)
TRS	total reducing sugars (g/L)

TS total sugar (g/L)
Y_{DXT} dextran yield (%)
Y_{Olig} oligosaccharide yield (%)

Statistical analyses

Results are shown as means±SD (three replicates). The data were statistically examined by analysis of variance at a 95 % of confidence level, using the statistical software Statistica (Statsoft) version 7.0.

Results and discussion

The clarified cashew apple juice presented a total content of 87.7 g/L total reducing sugars showing that it can be used as a source of acceptors (glucose and fructose) for the synthesis of prebiotics oligosaccharides. Table 1 presents the experimental

Fig. 1 Pareto chart of standardized effects of the independent variables on the dependent variables. **a** Dextran concentration (g/L); **b** Dextran yield (%); **c** oligosaccharides concentration (g/L); **d** oligosaccharides yield (%)

Table 2 Analysis of variance (ANOVA) for model validation (Eqs. 9 and 10)

Source of variation	Square sum	Degree of freedom	Mean square	F Value
Dextran yield (Equation 9)				
Regression	145.81	5	29.1	8.64
Residual	16.86	5	3.37	
Total	162.67	10		
R ²	0.89			
Listed F value (95 %)				5.05
Oligosaccharides (Equation 10)				
Regression	3773.21	5	754.64	5.82
Residual	648.71	5	129.74	
Total	4421.93	10		
R ²	0.85			
Listed F-value (95 %)				$F_{5,5}=5.05$

planning and the responses obtained: dextran; dextran yield (Y_{DXT}); oligosaccharides and oligosaccharides yield (Y_{Olig}). Figure 1 shows the pareto plots for the estimated effects of the independent variables on the studied responses. As shown in Fig. 1a, only the quadratic effect of sucrose was significant on dextran concentration. This effect was negative meaning that at high sucrose concentration dextran synthesis decreased. This occurs due to the substrate inhibition. According to Guimarães et al. (1999), at concentrations higher than 50 g/L substrate inhibition occurs. On dextran yield (Fig. 1b) both sucrose and reducing sugar linear effect were significant and positive, meaning that dextran yields increases linearly when reducing sugar or sucrose concentration is increased. On oligosaccharide concentration (Fig. 1c), only the reducing sugar quadratic effect was not significant and on oligosaccharide yield (Figs. 1d) all effects were non-significant.

The regression model obtained for dextran concentration and oligosaccharides yield were not statistically significant at 95 % of confidence level. Thus, response surface

methodology was not applied for these responses. Regression models for dextran yield and oligosaccharide concentration are presented in Eqs. 9 and 10, respectively.

$$Y_{DXT}(\%) = 8.80 + 0.35 \text{ Suc} - 5 \times 10^{-3} \text{ Suc}^2 + 0.03 \text{ RS} - 2 \times 10^{-3} \text{ RS}^2 + 1.1 \times 10^{-3} \text{ Suc} \times \text{RS} \quad (9)$$

$$\begin{aligned} \text{Oligosaccharides (g/L)} = & -2.69 + 1.11 \text{ Suc} \\ & + 2 \times 10^{-3} \text{ Suc}^2 + 0.30 \text{ RS} \\ & + 1.10 \times 10^{-3} \text{ RS}^2 \\ & + 2 \times 10^{-3} \text{ Suc} \times \text{RS} \end{aligned} \quad (10)$$

Where

Suc sucrose concentration (g/L)

RS reducing sugar (g/L)

Fig. 2 Contour surface: **a** Dextran yield (%), **b** oligosaccharides (g/L)

According to Table 2, which presents the ANOVA analysis for the regression models presented in Eqs. 9 and 10, the fitted model given in Eq. 9 is statistically significant because the calculated F-value (8.65) for the model of dextran yield was higher than the listed value ($F_{5, 5} = 5.05$) at 95 % of the confidence level. For the regression model presented in Eq. 10 (oligosaccharide concentration), the calculated F value was also higher than the listed one. Surface contour graphs obtained using the regression models are presented in Figure 2.

Figure 2a show that dextran yield is favored when concentrations in the range of 20 to 55 of sucrose and concentrations up to about 50 g/L of reducing sugar. High concentrations of sucrose and reducing sugars resulted in low yields of dextran. This behavior can be due a synergistic effect of the dextran synthesis suppression due to the acceptor reaction and some substrate inhibition. Figure 2b shows that oligosaccharide synthesis is favored when high concentration of sucrose and reducing sugar are applied. From Fig. 2, high concentration of acceptors suppressed the dextran synthesis and favored the oligosaccharides synthesis. Reducing sugars influenced the formation of oligosaccharides, because these sugars act as acceptors of the reaction of formation of prebiotic oligosaccharides. The highest concentration of oligosaccharides was obtained using 75 g/L sucrose in combination with 75 g/L of reducing sugars. At the same point the lowest concentration of dextran was obtained.

The degree of polymerization (DP) of the oligosaccharides is presented in Figure 3. Oligosaccharides with degrees of polymerization from 2 to 12 were obtained. Oligosaccharides synthesized by dextransucrase are homologous series (Heincke et al. 1999). Thus, the chain elongation depends on the availability of the shorter chain precursor. In all experimental runs oligosaccharides containing up to 5 glucose units (DP5) were obtained. Oligosaccharides with $DP > 5$ were obtained in Runs 1 to 4. In Run 2 (25.0 g/L sucrose and 75.0 g/L of reducing sugars, oligosaccharides with up to 12 glucose units were obtained. The use of partially purified enzyme allows the elongation of the oligosaccharide chain. Rabelo et al. (2009) and Vergara et al. (2010), produced oligosaccharides in cashew apple juice by fermentation of *L. mesenteroides* in cashew apple juice culture medium. These authors observed oligosaccharides with degree of polymerization up to 6 in cashew apple juice. Oligosaccharides concentrations found herein are in agreement to the obtained by Rabelo et al. (2006) for oligosaccharides synthesis using maltose as acceptor. Maltose is the strongest known acceptor of dextransucrase while fructose and glucose are considered weak acceptors (Heincke et al. 1999). It is not possible to compare our results with the results of other groups on oligosaccharides synthesis in fruit juices due to the lack of publication in this field. The use of the product for ready to drink formulations and as food ingredient for other products development is subject of further studies.

Fig. 3 Degree of polymerization of the oligosaccharides formed in cashew apple juice according to the experimental conditions presented in Table 1 (Runs 1 to 11)

Conclusions

The most commercial products containing oligosaccharides are obtained by the addition of oligosaccharides in the food matrix. These oligosaccharides are usually obtained by enzyme process (synthesis and/or hydrolyses) and have to be purified before the addition to the food matrix. The separation protocol usually increases the cost of the food ingredient resulting in higher costs to the consumer. Herein, we demonstrated that cashew apple juice is a suitable substrate for in vitro synthesis of oligosaccharides reducing the production costs. Besides representing an alternative to dairy based functional beverages, the process applied herein reduces the sugar content of the natural juice resulting in a low caloric product. Due to absence of yeast extract and other by-products obtained when fermentation process is used, cashew apple juice containing in vitro synthesized oligosaccharides can be used as ready as raw material to ready to drink beverage and or as food ingredient in other products.

Acknowledgments Acknowledgments The authors thank Conselho Nacional de Pesquisa de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support of this work and the ARS culture collection for providing the microbial strain.

References

- Chagas CMA, Honorato TL, Pinto GAS, Maia GA, Rodrigues S (2007) Dextranucrase production using cashew apple juice as substrate: effect of phosphate and yeast extract addition. *Bioprocess and Biosyst Eng* 30:207–215
- Dubois M, Gilles KA, Hamiltom PA, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. *Anal Chem* 28:350–356
- Fernando WMADB, Flint S, Zou M, Brennan CS, Ranaweera KKDS, Bamunuarachchi A (2011) The effect of rice fibre fractions on the growth of co-cultures of probiotics. *J Food Sci Tech* 48:14–25
- Fontes CPML, Honorato TL, Rabelo MC, Rodrigues S (2009) Kinetic study of mannitol production using cashew apple juice as substrate. *Bioprocess Biosys Eng* 32:493–499
- Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr-Bethesda* 125:1401–1412
- Guimarães DRB, Costa FAA, Rodrigues MI, Maugeri F (1999) Optimization of dextran synthesis and acidic hidrolisys by surface response analysis. *Braz J Chem Eng* 16:129–139
- Heincke K, Demuth B, Jördening HJ, Buchhols (1999) Kinetics of the dextranucrase acceptor with maltose – experimental results and modeling. *Enz Microb Technol* 24:523–534
- Honorato TL, Rodrigues S (2010) Dextranucrase stability in cashew apple juice. *Food Bioprocess Technol* 3:105–110
- Honorato TL, Rabelo MC, Gonçalves LRB, Pinto GAS, Rodrigues S (2007) Fermentation of cashew apple juice to produce high added value products. *World J Microbiol Biotechnol* 23:1509–1415
- Madhukumar M S, Muralikrishna G (2011) Fermentation of xylo-oligosaccharides obtained from wheat bran and Bengal gram husk by lactic acid bacteria and bifidobacteria. *J Food Sci Tech*, in press, DOI [10.1007/s13197-010-0266-7](https://doi.org/10.1007/s13197-010-0266-7).
- Manosroi J, Khositsunti Wong N, Manosroi A (2011) Biological activities of fructooligosaccharide (FOS)-containing *Coix lachryma-jobi* Linn extract. *J Food Sci Tech*, in press, DOI [10.1007/s13197-011-0498-6](https://doi.org/10.1007/s13197-011-0498-6).
- Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 31:426–428
- Rabelo MC, Honorato TL, Gonçalves LRB, Pinto GAS, Rodrigues S (2006) Enzymatic synthesis of prebiotic oligosaccharides. *App Biochem Biotechnol* 133:31–40
- Rabelo MC, Fontes CPML, Rodrigues S (2009) Enzyme synthesis of Oligosaccharides using cashew apple juice as substrate. *Biores Technol* 100:5574–5580
- Rodrigues S, Lona LMF, Franco TT (2003) Effect of phosphate concentration on the production of dextranucrase by *Leuconostoc mesenteroides* NRRL-B512F. *Bioprocess Biosys Eng* 26:57–62
- Rodrigues S, Lona LMF, Franco TT (2006) Optimizing panose production by modeling and simulation using factorial design and surface response analysis. *J Food Eng* 75:433–440
- Silveira MS, Fontes CPML, Guilherme AA, Fernandes FAN, Rodrigues S (2010) Cashew apple juice as substrate for lactic acid production. *Food Bioproc Technol*, in press, DOI [10.1007/s11947-010-0382-9](https://doi.org/10.1007/s11947-010-0382-9).
- Vergara CMA, Honorato TL, Maiga GA, Rodrigues S (2010) Prebiotic effect of fermented cashew apple (*Anacardium occidentale* L.) juice. *LWT - Food Sci Tech* 43:141–145