Biosynthesis of xanthan gum from pectin deriving from passion fruit peel extraction

ABSTRACT
Heteropolysaccharide obtained from the bacteria Xanthomonas fermentation, xanthan gum is widely used in the food, petrochemical, oil, agrochemical and pharmaceutical industries. This work evaluates the process of this polysaccharide production from pectin extracted from passion fruit peel, in the proportion of 1.5% (m/V), with and without sucrose supplementation, compared to the standard medium (YM). The fermentation process was carried out in an orbital shaker at 200 rpm and 28 °C for 72 hours. The gum yields obtained with pectin as carbon source were higher than that found with the standard medium, where with only pectin reached 13.83 g/L, while with pectin and sucrose it reached 18.87 g/L and in the standard medium the maximum yield was 9.76 g/L.

Keywords: Xanthomonas, passion fruit peel, yield, fermentation, pectin

RESUMO
O heteropolissacarídeo obtido da fermentação da bactéria Xanthomonas, goma xantana, é amplamente utilizado nas indústrias alimentícia, petroquímica, petrolífera, agroquímica e farmacêutica. Este trabalho avalia o processo dessa produção de polissacarídeos a partir de pectina extraída da casca de maracujá, na proporção de 1,5% (m / V), com e sem suplementação de sacarose, em comparação com o meio padrão (YM). O processo de fermentação foi realizado em um agitador orbital a 200 rpm e 28 °C por 72 horas. Os rendimentos de goma obtidos com a pectina como fonte de carbono foram superiores aos
encontrados no meio padrão, onde apenas a pectina atingiu 13,83 g / L, enquanto a pectina e sacarose atingiu 18,87 g / L e no meio padrão o rendimento máximo foi de 9,76 g / L.

**Palavras-chave:** Xanthomonas, casca de maracujá, produção, fermentação, pectina

### 1 INTRODUCTION

Passion fruit (*Passiflora edulis*) is a native Brazilian fruit, used for both fresh and industrial consumption, where the destination of a good part of the fruit is for the manufacture of juice and fruit pulp. Due to the great pectin content in its shell, passion fruit may become an alternative source for its extraction, usually produced in the Brazilian market from citrus albedo (Iglesias; Losano, 2004).

Pectin has a gelling, stabilizing and thickening effect on food and is also used as a substitute for sugar and fat in dietary foods (Iglesias; Lozano, 2004). According to Druzian & Pagliarini (2007), it can also be used in the production of xanthan gum, because the pectic polysaccharides promote viscosity increase.

Usually originated from corn glucose, xanthan gum is a heteropolysaccharide obtained from the fermentation of corn starch with the bacterium of the genus *Xanthomonas*, widely used in the food, pharmaceutical and hygiene and cosmetic industries, as stabilizer, emulsifier and thickener, as well as in the petrochemical industry. These bacteria are easy to cultivate in the laboratory, because they are aerobic and also microaerophilic, with an optimum temperature of growth between 25 and 30 °C. The exopolysaccharides produced by *Xanthomonas* can be obtained by fermentation in different culture media and substrates, with the most varied yields in relation to the volume and composition of the medium used (Druzian; Pagliarini, 2007).

In this paper, the feasibility of the use of pectin extracted from passion fruit peel for the production of xanthan gum is evaluated, since it is a polymer with a gelling action and can promote an increase in the viscosity of the gum at the same time as adding value to a waste product.

### 2 MATERIALS AND METHODS

The passion fruit peels, obtained after the juice processing, were cut, washed with water and sanitized with 100 ppm of sodium hypochlorite for 15 min. After sanitization, the residue was dehydrated in dryer with forced air circulation at 50 °C up to constant weight. After drying, the residues were crushed, sieved and stored at room temperature.
Pectin was extracted in citric acid at 90 °C, with a reaction time of 55 min and 5% (m/V) of residue, according to Canteri-Schemin et al. (2005) with some modifications. The dried pectin was employed as an alternative carbon source for the xanthan gum production.

The strain of *Xanthomonas campestris* pv. *manihotis* IBSBF 1182 was used in the experiments. This species was obtained from the Biological Institute of São Paulo. To obtain the inoculum, the strain was cultivated in yeast malt (YM) medium (0.3% malt extract, 0.3% yeast extract, 0.5% bacteriological peptone, and 1.0% glucose) and incubated at 28 °C for 24 h at 200 rpm.

The fermentations were conducted with YM and 1.5% (m/V) pectin as carbon source, without and with 1.5% (m/V) of sucrose, being added to 0.01% (w/v) urea and 0.1% (w/v) potassium phosphate, with the pH being adjusted to 7.0 and distributed in 250 mL Erlenmeyer flasks and sterilized at 121 °C for 15 min.

The fermentation started with the addition of 5% (V/V) of the inoculum and was conducted in an orbital shaker at 200 rpm for 72 h, with collections performed every 24 h. The sample was pasteurized at 90 °C for 15 min and separated from the cells by centrifugation at 7500 rpm and 20 min at 4 °C. Cell growth, total reducing sugars (TRS) and xanthan gum production (product) were evaluated. These results were expressed by the average ± standard deviation of triplicate measure. Cell growth was determined by measuring optical density of cells at 560 nm in the fermentation broth and correlated with dry cell weight (105°C until constant weight). The total reducing sugars (TRS) were determined by the DNS colorimetric method (Miller, 1959), after hydrolysis of the extract with H₂SO₄ 1.5 M and neutralization with NaOH 2 N (Silva et al., 2015).

The polymer was recovered by precipitation from the supernatants by adding 96 °GGL ethanol (1:2). The gum samples were separated and transferred to plates, dried at 50 °C for 24 h and stored in a sealed flask for analysis. The yield from each residue was calculated and the values were expressed in g/L (grams of gum per liter of culture medium) (Costa et al., 2014).

3 RESULTS AND DISCUSSION

In order to evaluate the use of passion fruit peel pectin as an alternative carbon source for the production of xanthan gum, a simultaneous fermentation with the standard medium (YM) was carried out, with the results shown in Figure 1.
In the pectin trials, the *Xanthomonas* showed maximum growth in 48 h, while in the YM medium had a linear growth during the 72 h. As for the substrate consumption, the pectin assays had increases and decreases in consumption over 72 h, which may be due to substrate hydrolysis during fermentation. The maximum yield occurred at 48 h in pectin trials, generating 13.83 g/L and 18.87 g/L in the experiments without and with sucrose, respectively. In YM, the maximum yield was reached at 72 h (9.76 g/L), 30% less than the pectin-only test as a source of carbon, indicating that the use of pectin as an alternative source for the production of xanthan gum is very feasible.

With the same strain of *Xanthomonas*, Diniz et al. (2012) obtained a xanthan gum yield with cocoa husk residues of 7.34 g/L, and with whey 12.01 g/L, in comparison to sucrose as carbon source (2.42 g/L), after 120 h of fermentation. The results using pectin were found to be more satisfactory, indicating the need to improve the process conditions, as also to obtain a better characterization of the gum obtained in relation to the commercial gum, from analysis of viscosity, color, X-ray diffraction, among others.
4 CONCLUSIONS

Pectin from the passion fruit peel extraction proved to be a good alternative for the production of xanthan gum, obtaining a good gum yield. The addition of sucrose had a significant influence on the yield of the gum, increasing in around 5 g/L in comparison to the experiment with only pectin. The maximum yields of xanthan gum occurred at 48 h in the experiments with pectin (13.83 g/L without sucrose and 18.87 g/L with sucrose) and in 72 h with the standard medium (10.79 g/L).

ACKNOWLEDGMENTS

The authors are grateful for the PIBIC-UFS program and the CNPq for the scholarship.

REFERENCES

Canteri-Schemin, M. H., FertonanI, H. C. R., Waszczynskyj, N., Wosiacki, G. Extraction of pectin from apple pomace. Brazilian Archives of Biology and Technology, v. 48, n. 2, p. 259-266, 2005.

Costa, L.A.S., Campos, M.I., Druzian, J.I., Oliveira, A.M., Oliveira, Junior, E.N. Biosynthesis of xanthan gum from fermenting shrimp shell: yield and apparent viscosity. International Journal of Polymer Science, p.1-9, 2014.

Diniz, D. M., Druzian, J. I., Audibert, S. Produção de goma xantana por cepas nativas de Xanthomonas campestris a partir de casca de cacau ou soro de leite. Polímeros, v. 22, n. 3, p. 278-281, 2012.

Druzian, J. I., Pagliarini, A. P. Produção de goma xantana por fermentação do resíduo de suco de maçã. Ciência e Tecnologia de Alimentos, v. 27, n. 1, p. 26-31, 2007.

Iglesias, M. T., Lozano, J. E. Extraction and characterization of sunflower pectin. Journal of Food Engineering, v. 62, n. 3, p. 215-223, 2004.

Miller, G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, v. 31, n. 3, p. 426-428, 1959.

Silva, C.E.F., Gois, G.N.S.B., Silva, L.M.O., Almeida, R.M.R.G., Abud, A.K.S. Citric waste saccharification under different chemical treatments. Acta Scientiarum Technology, v. 37, n. 4, p. 387-395, 2015.