BIOSYNTHESIS OF XANTHAN GUM BY XANTHOMONAS CAMPESTRIS USING RICOTTA WHEY AS SUBSTRATE

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ABSTRACT

Xanthan gum is a biopolymer obtained through the cultivation of the bacterium *Xanthomonas campestris*, which has thickening and stabilizing properties. The aim of this research was to determine the ideal conditions for the production of xanthan gum from ricotta whey lactose, a by-product of the dairy industry. For this, a DCCR experimental design with two variables was used, where the concentration of serum and urea was varied, subjecting the mixture to cultivation in a shaker for 120 hours at 28°C and 200 rpm. By analyzing the yield and viscosity obtained in each experiment, it was possible to identify the optimal production conditions. The optimal conditions were found to be 0.75% (w/v) of urea and 60% (v/v) of ricotta whey, which produced 0.914 g/L of xanthan gum at its peak.

Keywords: Xanthan gum, biopolymer, ricotta, yield.

1 INTRODUCTION

Xanthan gum, produced by bacteria of the genus Xanthomonas sp., is a promising biopolymer to replace conventional polymers.[1] Used in the food and pharmaceutical industries, it functions as a thickener, emulsifier and dispersant, standing out for its rheological properties, such as high viscosity and solubility in water.[2] These properties are determined by their chemical composition and structure.[3] Production involves several steps, including fermentation and dehydration, with the final quality depending on the substrate and carbohydrate availability.[4] In Brazil, xanthan gum is mainly imported, which increases costs and market vulnerability. A viable alternative is to use agro-industrial waste, such as lactose-rich ricotta whey, as a carbon source, transforming combustible waste into significant products that avoid environmental impact.[5] Bioprocess engineering is of paramount importance in the development of sustainable biotechnological processes. By aligning economic and environmental objectives, it is possible to create a more competitive and sustainable industry. The objective of this study is to identify the optimal conditions for the production of xanthan gum, with a focus on maximizing yield and viscosity.

2 MATERIAL & METHODS

The experiment was conducted using ricotta whey obtained from the Menininha do Campo dairy in Conselheiro Lafaiete, Minas Gerais, Brazil. A YM medium containing 1% glucose, 0.5% bacteriological peptone and 0.3% meat extract and 0.3% yeast extract was prepared. After autoclaving this medium, 1 cryotube containing Xantomonas Campestris was added and incubated in an orbital shaker at 250 rpm and 28°C for 24 hours. Moreover, a fractional DCCR 2^2 statistical planning was carried out with 4 axial points and quintuplicate at the central point to evaluate the production and preparation conditions of the media, which is depicted in Table 1.[5] The amount of 0.1g of $MgSO_4 \cdot 7H_2O$ was added to each test in a final volume of 100 ml. Subsequently, the media was sterilized and cooled, and the bacteria were reactivated after 24 hours on the orbital shaker. Each erlenmeyer flask was inoculated with 10 mL of inoculum in a laminar flow hood to ensure asepsis. Then, the inoculum media were agitated at 28°C and 250 rpm for 120 hours to produce xanthan gum. After this period, centrifugation was performed at 4800 rpm and 4°C for 20 minutes to separate the residues.

Table 1 The matrix of the central composite rotatable design, which includes the real and coded values (in parentheses) for the response gum yield.

Run	Urea (% w/v)	Ricotta whey (% v/v)	Yield (g/L)	Viscosity (mPa.s)
1	0.22 (-1)	39 (-1)	0	0
2	0.22 (-1)	81 (+1)	0.756	3.64
3	1.28 (+1)	39 (-1)	0.495	3.22
4	1.28 (+1)	81 (+1)	0.717	2.70
5	0 (-1.41)	60 (0)	0.726	2.82
6	1.5 (+1.41)	60 (0)	0.629	2.58
7	0.75 (0)	30 (-1.41)	0.543	3.30
8	0.75 (0)	90 (+1,41)	0.789	2.43
9*	0.75 (0)	60 (0)	0.914	3.40
10*	0.75 (0)	60 (0)	0.828	3.05
11*	0.75 (0)	60 (0)	0.862	3.40

Furthermore, 92.8° GL ethyl alcohol was added to precipitate the gum, followed by filtration with a commercial sieve. Then, the gum was frozen, freeze-dried, crushed and stored for further analysis. To determine the specificity of xanthan gums, 0.1% (w/v) solutions were prepared and stirred for 12 hours at 25°C. Viscosity was measured at 60 rpm and 25°C using a Brookfield DV-II viscometer with a concentric cylinder device coupled to a water bath. The results of analysis and figures were obtained by using the software Statistica.[6]

3 RESULTS & DISCUSSION

According to Table 1, the lowest and highest values for the production of xanthan gum from ricotta whey were 0.495 g.L-1 in test 3 and 0.914 g.L-1 in test 9. The results of this study are comparable to those of other studies in terms of behavior and proportion, despite differences in the bacterial strains used. Other studies have employed a mixed culture, whereas this study has used a single culture.[7] Although literature have reported high yields with this substrate, our research group focused on avoiding the precipitation of other substrates along with xanthan gum, which would increase the yield but result in a "false yield".

In Figure 1 it is possible to observe the significance of each variable through the Pareto charts and the contour diagrams based on the results found.

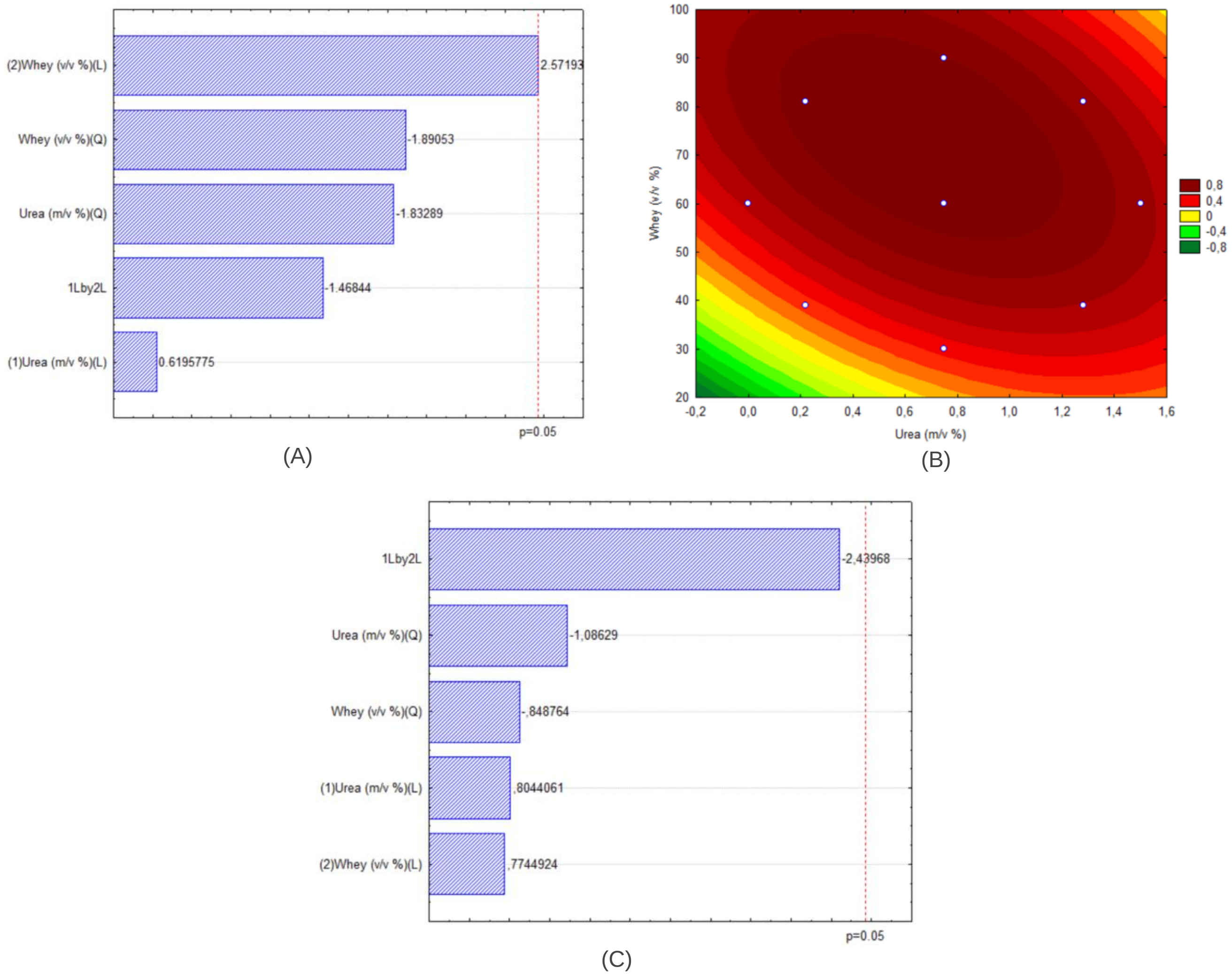

Figure 1 - Pareto chart of ricotta whey and urea effects on the gum yield (A), contour diagram of gum yield (%) as a function of ricotta whey v/v (%) and urea (w/v %) (B) and Pareto chart of ricotta whey and urea effects on the gum viscosity (C).

The Pareto diagram enabled us to identify the significance of variables in the process. Moreover, the most statistically significant effect was that of whey on gum yield, with a p-value of less than 0.05, as indicated in Figure 1A. The central point exhibited the most promising conditions, with the highest yields, situated in the darkest region of the surface, which indicated its excellent performance, as depicted in Figure 1B. Additionally, the mean yield observed at the central point was 0.868 g/L, while the lowest yield was observed in run 3, with a yield of 0.495 g/L.

In the case of effects on gum viscosities, both whey and urea were not found to be statistically significant. In Figure 1C, it is observed that all the effects were higher than p=0.05 and for this reason the contour diagram of gum viscosity as a function of ricotta whey and urea was not showed.

A positive aspect was observed in the apparent viscosity, with particular emphasis on the central points, which exhibited viscosities that were only lower than those of Test 2. In contrast to the existing literature, this study identified xanthan gum with low apparent viscosity, which is of significant interest as it facilitates the industrial use of large polymers, which are often challenging to handle. This discovery opens the door to exploring ideal applications for polymers with this characteristic.

The response surface indicates that high yields were observed in several regions. However, it is notable that the central points in the optimum region exhibit a significant prominence.

4 CONCLUSION

It was demonstrated that xanthan gum could be produced using ricotta whey, with the optimal production and viscosity conditions occurring, for the most part, in the central points. The low apparent viscosity observed in this study does not represent a problem, but rather a desirable characteristic for several industrial applications dealing with polymers. However, the yields obtained were very low compared to the literature, both with the same substrate and with different substrates. This makes the project unfeasible on larger scales at the moment; however, further research will be conducted to improve the feasibility.

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