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Production of Xanthan Gum from Inedible Parts of Broccoli and Cauliflower

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The cost of producing xanthan gum by *Xanthomonas campestris* is heavily impacted by the use of sugar or dextrose as a carbon source from an industrial standpoint. To address this, the researchers in this study opted to use kitchen waste, a substantial solid waste from the food industry, as a valuable source of fermentable sugars. Inedible parts of broccoli and cauliflower used as kitchen waste in this study show promising potential as an economically and ecologically sustainable material for fermenting biomolecules. This study aims to evaluate the viability of utilizing kitchen waste as a cost-effective, ecologically sustainable carbon source to produce xanthan, making use of *Xanthomonas campestris NCIM 2961* in the process. Aqueous extracts of inedible parts of cauliflower and broccoli were fermented with *Xanthomonas campestris NCIM 2961* at standard conditions. The fermentation parameters, including, pH, temperature, agitation, and incubation period were varied at different levels to study the effects of varying conditions on the xanthan yield and to determine the optimum levels of the fermentation parameters. After the fermentation process, the xanthan gum was separated from the broth through alcoholic precipitation and subsequent drying. The weight of the dried gum was recorded. To analyze the properties of the xanthan obtained from the alternative medium under standard conditions, it was compared with commercial food-grade xanthan using Fourier-transform infrared (FTIR) spectroscopy. The FTIR spectra of xanthan produced from the alternate medium showed a close resemblance to that of the commercial food-grade xanthan. The results obtained validate the potential of kitchen waste as a cost-effective, and eco-friendly alternative carbon source for xanthan production, thereby decreasing the cost of production and solid waste generated.

Keywords: xanthan gum, waste management, kitchen waste, ecological sustainability.

Introduction

Xanthan gum constitutes a biopolymer derived through the fermentation of diverse *X. campestris* strains under appropriate reaction conditions. Its remarkable attributes include exceptional solubility, thermal resistance, pH stability, high viscosity, pseudoplasticity, and cross-linking abilities, rendering it a highly desirable option for numerous industrial uses, particularly in the food and cosmetic sectors (Palaniraj and Jayaraman, 2011). *X. campestris*, among the different *Xanthomonas* strains, showcased superior efficiency, achieving

a conversion rate of around 80% which consequently makes it a widely favored option for xanthan production (Lei and Edmund, 2017). However, the industrial manufacture of xanthan using conventional carbon sources is very expensive and consequently increases the overall production costs and results in the exorbitant pricing of the end product. This creates a necessity to investigate an economical alternative carbon source. Over the years, numerous researchers have conducted experiments involving diverse agricultural wastes and lignocellulosic substrates with the intention of reducing the manufacturing costs of xanthan. Date palm juice (*Phoenix dactylifera*) by-products have been explored as a substrate for xanthan fermentation, utilizing *X. campestris NRRL B-1459*, in an effort to reduce the production cost (Salah et al., 2011). Ozdal and Kurbanoglu (2019) have employed sugar beet molasses enriched with 4 g/L of chicken feather peptone (CFP) as an alternative substrate, resulting in a maximum xanthan yield of 20.5 g/L after 60 hours of incubation, with complete sugar consumption. Jesus et al. (2014) have developed an alternative medium for xanthan gum production using hemicellulosic fractions of corncob, involving grinding dried corncob, alkaline pre-treatment and subsequent incubation at 180 rpm for 96 hours at 28°C following inoculation. The highest xanthan production after incubation was reported to be 11.7 g/L/h (Jesus et al., 2014). Li et al. (2016) have investigated the feasibility of xanthan production by fermentation using acid-hydrolyzed kitchen waste as the sole substrate at different dilutions, and the concentrations of xanthan gum obtained at the end of 96 h of batch fermentation have been found to be 9.56 g/L and 10.94 g/L in undiluted and diluted kitchen waste hydrolysate, respectively. A maximum xanthan yield of 10.03 g/L has been obtained using confectionery wastewater as fermentation media enriched with sucrose and yeast extract (Bajić et al., 2014).

Similar results have been obtained using rose wine and white wine effluents from wine industry as an alternative substrate for xanthan production (Rončević et al., 2017). In another study, maximum yields of 4.48 g/L and 3.89 g/L have been found using cocoa husks minimally supplemented with urea and potassium using the strains *X. campestris pv. campestris 1866* and *X. campestris pv. campestris 1867* (Da Silva et al., 2018). Demirci et al. (2019) have utilized waste bread hydrolysate to biosynthesize xanthan gum and obtained a highest yield of 14.3 g/L using *X. axonopodis pv. vesicatoria*. Soleymanpour et al. (2018) have used alkali pretreated and subsequent acid hydrolysed broomcorn stem as an alternative substrate and achieved a maximum concentration of 8.9 g/L of xanthan gum. Alkali pretreated potato waste has been utilized by (Sirohi and Kumar, 2019) as a carbon source to achieve a maximum xanthan yield of 45 g/L after 96 h of incubation. Jackfruit seed powder has been used as a novel substrate supplemented with peptone, citric acid, K_2HPO_4, KH_2PO_4, K_2HPO_4, and KH_2PO_4 to produce a maximum yield of 51.62 g/L (Felicia Katherine et al., 2017). Purwadi et al. (2009) have used cabbage extract as a precursor in xanthan gum production using *X. campestris* supplemented with a yeast extract to achieve a maximum concentration of 12 g/L of xanthan gum. The main objective of this study was to evaluate the ability of the inedible parts of broccoli and cauliflower, usually discarded as a part of kitchen waste, to serve as a cost-effective and eco-friendly carbon source for xanthan fermentation. Based on the data obtained from the Food and Agricultural Organization of the United Nations (FAOSTAT) Statistics Division, the combined production of cauliflower and broccoli has shown an exponential increase over the past two decades. The quantities of cauliflower and broccoli produced have risen by 27.3% from 2001 to 2010 and experienced a substantial 38.1% increase from 2001 to 2021. This significant growth highlights the considerable amount of solid waste generated from the extensive production of cruciferous vegetables like cauliflower and broccoli. Considering the potential of solid kitchen waste as a sustainable, eco-friendly substrate, the authors designed a study to evaluate the viability of inedible parts of broccoli and cauliflower as the substrate to produce xanthan.

Materials and Methods

Media and bacterial strain

A pure bacterial culture of *X. campestris NCIM 2961* was sourced from the National Collection of Industrial Microorganisms (NCIM), Pune, and used for all the experiments throughout this study. An MGYP medium (malt extract = 0.3 g/L, glucose = 1 g/L, yeast extract = 0.3 g/L, and peptone = 0.5 g/L in 100 mL of distilled water) was used as the culture medium. The bacterial culture was subcultured at regular intervals in MGYP agar plates (2% agar-agar) to avoid culture degradation and maintain consistency in the experiments.

Liquid media culture

Standard MGYP medium

100 mL of the MGYP liquid medium was prepared in distilled water to serve as the standard medium. The pH of the prepared medium was adjusted to 6.8 using 1N HCl and 1N NaOH solutions. The medium was then divided equally into two parts (50 mL each) in two Erlenmeyer flasks and autoclaved prior to inoculation and subsequent fermentation.

Alternative medium

The alternative medium was prepared by homogenizing 50 g of the inedible parts of cauliflower and broccoli separately in 300 mL distilled water using a mixer-grinder (Inalsa). Following this, the extract was filtered using a muslin cloth. The pH of the medium was then adjusted to 6.8 using 1N HCl and 1N NaOH solutions and divided equally into 50 mL aliquots and transferred to six Erlenmeyer flasks and sterilized via autoclaving prior to fermentation. Furthermore, the reducing sugar content in the alternative medium was determined using the DNSA method of reducing sugar estimation.

Xanthan recovery

Following incubation, the biomass was separated from the fermentation broth using a cooling centrifuge (Remi C-24BL) at 5000 rpm for 2 minutes in 100 mL polypropylene centrifuge tubes. The precipitate was discarded, and the remaining supernatant was mixed with double the volume of isopropyl alcohol (IPA) for alcoholic precipitation. After cooling in the refrigerator for 1 hour, the mixture was centrifuged at 10 000 rpm for 10 minutes, effectively isolating the biopolymer from the fermentation broth. After centrifugation, the supernatant was discarded, and the pellet containing the xanthan gum was dried in a hot air oven at 70°C overnight. The dried xanthan gum was then weighed using an analytical weighing balance (Mettler Toledo), and the weight was recorded.

Variation of fermentation parameters

To determine the best conditions for xanthan production, the study explored the impact of varying agitation and incubation time through a series of experiments. Different levels of each parameter were tested to maximize the production of xanthan. For the initial experimentation, the aqueous extract of cauliflower and broccoli was divided into 50 mL aliquots, and the pH levels were adjusted to 6.0, 6.8, and 7.0. These medium samples were then inoculated and incubated for 24 hours at 32°C with agitation at 100 rpm. The most effective pH level was determined based on the highest yield of the biopolymer. Next, to refine the temperature for maximal xanthan production from cauliflower and broccoli, the fermentation of cauliflower and broccoli extract was carried out at three different temperature levels: 28°C, 30°C, and 32°C, while maintaining the pH at the previously identified best level (pH = 6.8). For enhancing mass transfer in the medium, agitation was varied at 100 rpm, 150 rpm, and 200 rpm at a constant temperature of 32°C, pH of 6.8, and an incubation time of 24 hours and to achieve the highest xanthan yield, the incubation time for fermentation of BSG extract was adjusted at three levels: 24 hours, 48 hours, and 72 hours, while keeping the temperature at 32°C, agitation at 100 rpm, and pH at 6.8.

Characterization of xanthan produced from Alternative fermentation media

Samples of commercial food-grade xanthan gum (Urban Platter Professional Xanthan Gum Powder, Mumbai, India) and xanthan gum obtained from aqueous extracts of broccoli and cauliflower parts were powdered and subjected to FTIR at Guwahati Biotech Park Incubation Centre for spectral analysis. The FTIR was performed on K-Br pellets of the samples using Thermo Nicolet iS10 FTIR Spectrometer (Thermo Scientific).

Results and Discussion

Reducing sugar estimation by DNSA method

The concentration of reducing sugars in the broccoli-based medium and the cauliflower-based medium were estimated to be 16.5 g/L and 5.4 g/L after analysis.

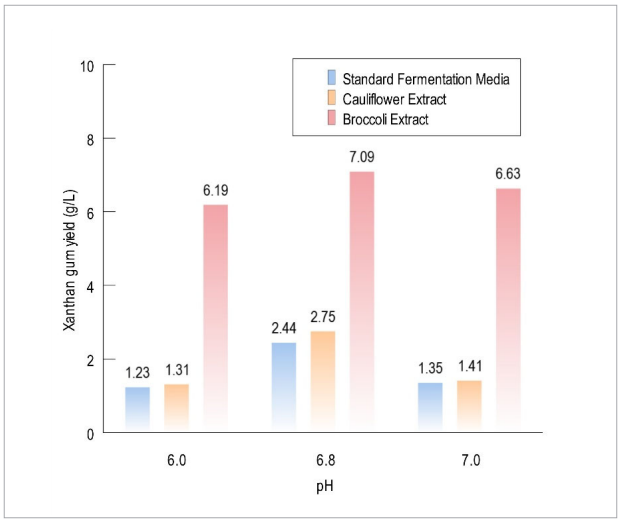
Evaluation of fermentation parameters at varied levels to achieve maximum xanthan production

Effects of pH variation on xanthan gum yield from liquid fermentation media

Experiments using *X. campestris* NCIM 2961 revealed the maximal yield in the standard MGYP medium at 32°C, 100 rpm agitation to be at pH = 6.8. However, the most effective pH for the broccoli- and cauliflower-based media requires pH optimization for which the pH was varied at three different levels (6.8, 6.0, and 7.0) to assess the ideal pH. In addition, the standard MGYP medium fermentations were also carried out at different pH levels to compare with that of the alternative medium. The maximum xanthan yield obtained from the cauliflower-based

medium, the broccoli-based medium, and the MGYP medium were 2.75 g/L, 7.09 g/L and 2.44 g/L, respectively, at a pH of 6.8, 32°C (*Fig. 1*), continuous agitation at 100 rpm after 24 h of incubation which indicates a 11% increase in xanthan yield from the cauliflower-based medium relative to the control MGYP medium and an impressive 65% increase in xanthan yield compared with the yield from the control MGYP medium.

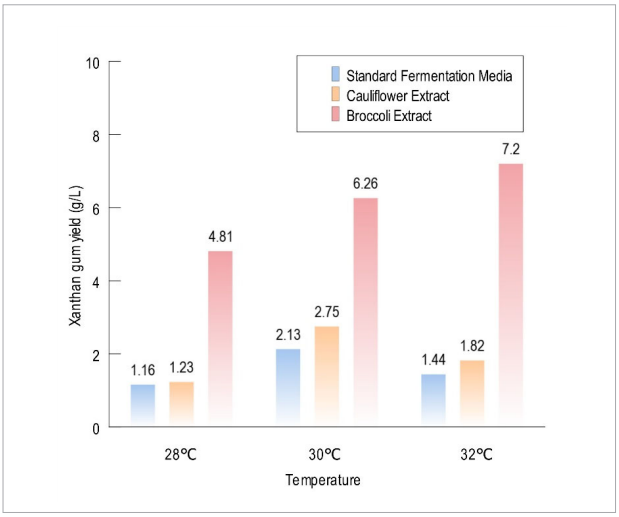
Fig. 1. *Xanthan gum yields from standard fermentation medium, cauliflower-based medium (cauliflower extract), and broccoli-based medium (broccoli extract) at varying pH levels*



Effects of temperature variation on xanthan gum yield from liquid fermentation media

The temperature for fermentation of the cauliflower-based medium, the broccoli-based medium and the standard MGYP medium was varied in three levels to determine the most effective temperature for the highest xanthan yield. The average yield of xanthan was calculated at different temperatures. The maximum yield of xanthan gum from the cauliflower-based medium and the broccoli-based medium were observed at 30°C and 32°C, respectively. Xanthan yield increased steadily with the increase in temperature from 28°C to 30°C at pH = 6.8. The maximum xanthan gum yield was 2.75 g/L observed at 30°C from cauliflower medium fermentation and 7.20 g/L observed at 32°C from the broccoli-based medium fermentation (*Fig. 2*). However, the highest xanthan yield from standard MGYP (2.13 g/L) was observed at 30°C. Comparing the results, xanthan gum obtained from the broccoli-based medium exhibited approximately a 70% increase in production compared with that from the standard MGYP medium, while the xanthan yield from the cauliflower-based medium showed an approximate 22% increment compared with the xanthan yield from the standard MGYP medium.

Fig. 2. *Xanthan gum yields from standard fermentation medium, cauliflower-based medium (cauliflower extract), and broccoli-based medium (broccoli extract) at varying temperatures*



Effects of agitation on xanthan gum yield from liquid fermentation media

Agitation during the fermentation of the broccoli- and cauliflower-based medium was varied at three different rates – 100 rpm, 150 rpm, and 200 rpm – to find the most effective agitation rate for the maximum xanthan yield. The greatest xanthan yield was observed at an agitation rate of 100 rpm at 32°C, pH = 6.8, and an incubation period of 24 hours for the cauliflower-based medium. However, the highest xanthan yield from the broccoli-based medium was observed at an agitation rate of 200 rpm at 32°C, pH = 6.8. The xanthan yield in the standard medium achieved a maximum yield of 2.13 g/L at a continuous agitation of 150 rpm, 32°C, pH = 6.8. However, the xanthan yield from the broccoli-based medium remained consistent and showed no significant variation under different levels of agitation, as indicated in *Fig. 3*. When comparing the results, the xanthan obtained from the cauliflower-based medium showed a 22% increase in the yield compared with the xanthan obtained from the MGYP medium (2.13 g/L). Conversely, the xanthan recovered from the broccoli-based medium (7.29 g/L) showed a 70% increase in the end-product relative to that obtained from the standard MGYP medium.

Fig. 3. *Xanthan gum yields from standard fermentation medium, cauliflower-based medium (cauliflower extract), and broccoli-based medium (broccoli extract) at varying agitation rates*

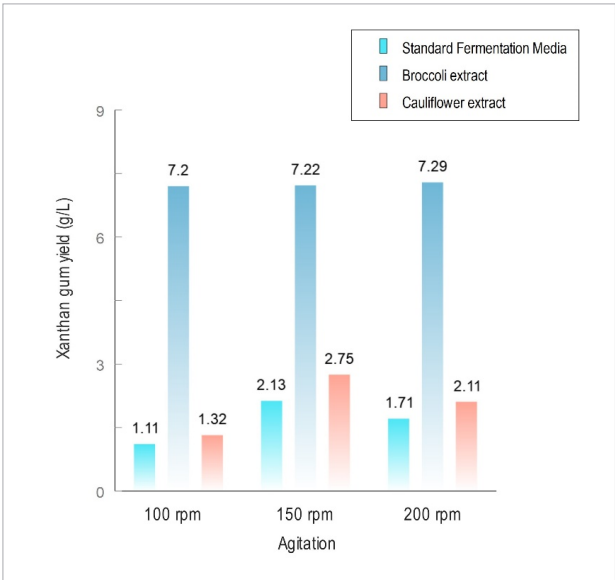
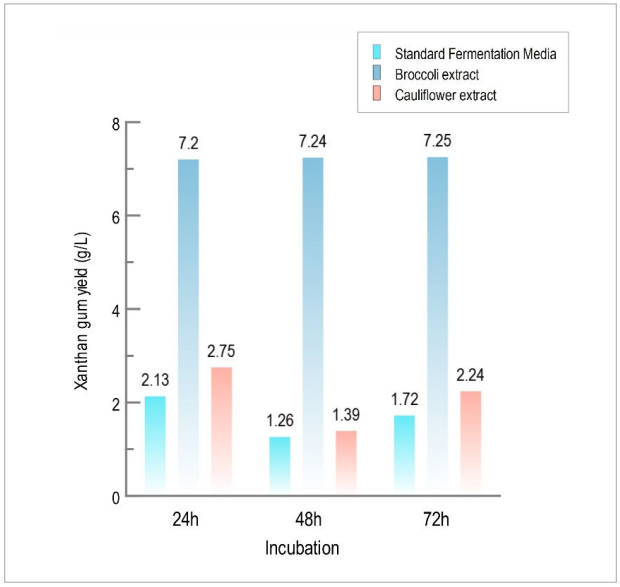


Fig. 4. *Xanthan gum yields from standard fermentation medium, cauliflower-based medium (cauliflower extract), and broccoli-based medium (broccoli extract) at varying incubation periods*



Effects of incubation period on xanthan gum yield from liquid fermentation medium

The incubation period of the fermentation of the broccoli-based medium and cauliflower-based medium were varied at three different levels – 24 h, 48 h, and 72 h. The highest xanthan yield from the broccoli-based medium (7.25 g/L) and the cauliflower-based medium (2.75 g/L) were observed during fermentation at 72 h and 24 h respectively. Notably, the xanthan yield from the broccoli-based medium remained consistent with no significant variation under different incubation time periods as indicated in *Fig. 4*. The highest xanthan yield from the standard MGYP medium (2.13 g/L) was observed at 24 h. After the completion of the variation experiments, the optimal parameters were obtained for the maximum production of xanthan gum from the broccoli-based medium and the cauliflower-based medium. *Table 1* depicts the best reaction conditions for the highest end-product yield from the MGYP medium and the alternative medium.

Table 1. *Most effective levels of the reaction parameters obtained for standard and alternative media*

Reaction parameters	Standard MGYP medium	Broccoli-based medium	Cauliflower-based medium
pH	6.8	6.8	6.8
Temperature	30°C	32°C	30°C
Agitation	150 rpm	200 rpm	150 rpm
Incubation period	24 h	72 h	24 h

Analysis of xanthan gum using FTIR for its characteristics

The FTIR spectra of the xanthan gum obtained from the cauliflower-based medium and the broccoli-based medium were compared with that of commercial food-grade xanthan gum (Urban Platter Professional Xanthan Gum Powder) to validate the results. The results of the analysis were interpreted (FTIR reference chart available as follows - IR Absorption Spectra Table).

There are many peaks in the spectral graph which shows that the analyzed compound is a complex chemical compound. The peaks contain a single bond area (2500–4000 cm^{-1}). No other peaks were found between 3000 and 3200 cm^{-1} indicating the absence of any aromatic groups. No specific peak for aldehyde was found between 2700 and 2800 cm^{-1}. No C triple bond region was found between 2000 to 2500 cm^{-1}. Regarding the double bond region (1500 to 2000 cm^{-1}), a sharp peak was detected at 1634.51 cm^{-1} for xanthan obtained from the broccoli-based medium and 1633.65 cm^{-1} for

xanthan recovered from the cauliflower-based medium, which indicates the presence of carbonyl double bonds. In the fingerprint region (600 to 1500 cm^{-1}), multiple signals were detected at 791.50 cm^{-1},1020.32 cm^{-1}, 1240.36 cm^{-1}, and 1403.57 cm^{-1} for xanthan extracted from the broccoli-based medium and 885.69 cm^{-1}, 1027.27 cm^{-1}, 1238.92 cm^{-1}, and 1404.01 cm^{-1}. Spectral data from FTIR of commercial food-grade xanthan show close resemblance with xanthan extracted from the cauliflower- and broccoli-based medium as depicted in *Figs. 5–7*. As a result, xanthan from the cauliflower- and broccoli-based medium and standard commercial xanthan gum have almost identical spectral characteristics that validate their identity.

Fig. 5. *FTIR spectra of commercial food-grade xanthan*

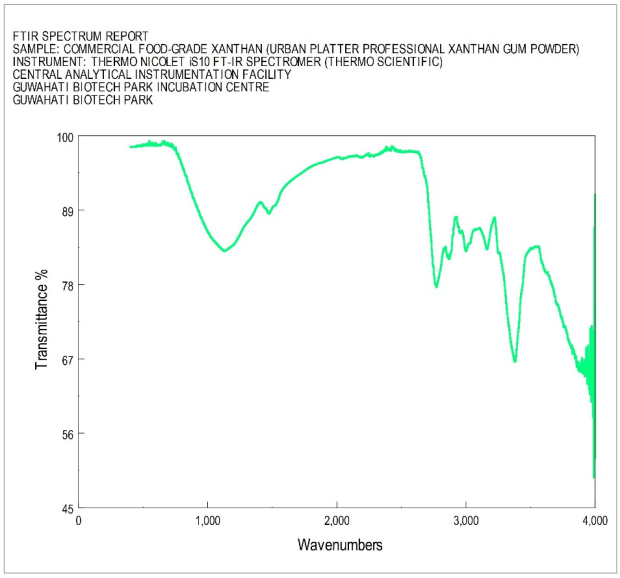


Fig. 7. *FTIR spectra of xanthan extracted from the cauliflower-based medium*

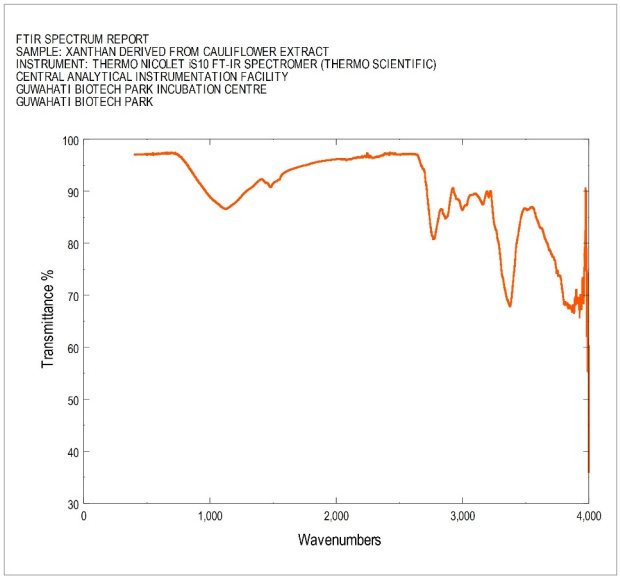
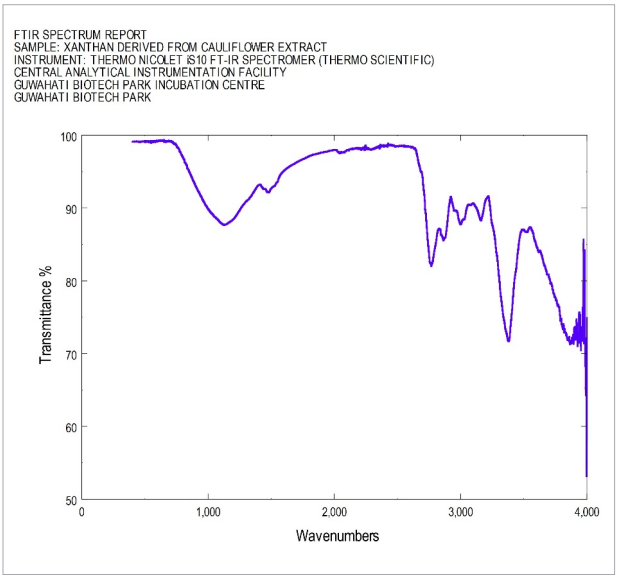


Fig. 6. *FTIR spectra of xanthan extracted from the broccoli-based medium*



Conclusions

Agricultural growth leads to an increase in solid waste generation, posing significant challenges in its management. However, an innovative solution to this problem lies in utilizing solid waste as a fermentative substrate in the industrial production of biomolecules. This approach not only addresses waste management but also reduces production costs and ultimately lowers the price of the end product. The results obtained from this study validate the effectiveness of using inedible parts of broccoli and cauliflower as an alternative carbon source to produce xanthan gum. The alternative medium used in the fermentation process produced a higher yield of xanthan gum compared with conventional MGYP medium in all experimental conditions, without requiring any input of nutritional supplements. The spectral data obtained from the FTIR characterization of xanthan extracted from the broccoli-based medium, cauliflower-based medium, and commercial food-grade xanthan gum exhibit close resemblance, which establishes the purity of the xanthan produced from alternative media. The findings in this

study confirm the viability of utilizing inedible parts of broccoli and cauliflower as a sustainable, ecological, and cost-effective alternative substrate for aerobic fermentation with *X. campestris NCIM 2961* at optimum reaction conditions. By adopting this approach, the issue of solid waste management associated with agricultural production can be effectively addressed. Rather than converting most of the waste into compost or animal feed, this study presents an eco-friendly and cost-effective alternative, which adds industrial value by reducing the production costs of xanthan.

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References

Bajić B. et al. (2014) Biosynthesis of xanthan gum on wastewater from confectionary industry. Analecta 8(2): 13-17. Available at: https://doi.org/10.14232/analecta.2014.2.13-17

Da Silva J. A., Cardoso L. G., De Jesus Assis D., Gomes G. V. P., Oliveira M. B. P. P., De Souza C. O., and Druzian J. I. (2018) Xanthan Gum Production by Xanthomonas campestris pv. campestris IBSBF 1866 and 1867 from Lignocellulosic Agroindustrial Wastes. Applied Biochemistry and Biotechnology 186(3): 750-763. Available at: https://doi.org/10.1007/s12010-018-2765-8

Demirci A. Ş., Palabiyik I., Apaydin D., Mirik M., and Gumus T. (2019) Xanthan gum biosynthesis using Xanthomonas isolates from waste bread: Process optimization and fermentation kinetics. Lebensmittel-Wissenschaft and Technologie 101: 40-47. Available at: https://doi.org/10.1016/j.lwt.2018.11.018

Felicia Katherine R., Muthukumaran C., Sharmila G., Manoj Kumar N., Tamilarasan K., and Jaiganesh R. (2017) Xanthan gum production using jackfruit-seed-powder-based medium: optimization and characterization. 3 Biotech 7(4). Available at: https://doi.org/10.1007/s13205-017-0876-5

Jesus M., Mata F., Batista R. A., Ruzene D. S., Albuquerque-Júnior R., Cardoso J. C., Vaz-Velho M., Pires P., Padilha F. F., and Silva D. P. (2023) Corncob as Carbon Source in the Production of Xanthan Gum in Different Strains Xanthomonas sp. Sustainability 15(3): 2287. Available at: https://doi.org/10.3390/su15032287

Lei S., and Edmund T. F. (2017) Polysaccharides, Microbial ☆. Encyclopedia of Microbiology (Fourth Edition). Available at: https://doi.org/10.1016/B978-0-12-809633-8.13102-4

Li P., Li T., Zeng Y., Li X., Jiang X., Wang Y., Xie T., and Zhang Y. (2016) Biosynthesis of xanthan gum by Xanthomonas campestris LRELP-1 using kitchen waste as the sole substrate. Carbohydrate Polymers 151: 684-691. Available at: https://doi.org/10.1016/j.carbpol.2016.06.017

Ozdal M., and Kurbanoglu E. B. (2019) Use of Chicken Feather Peptone and Sugar Beet Molasses as Low Cost Substrates for Xanthan Production by Xanthomonas campestris MO-03. Fermentation 5(1): 9. Available at: https://doi.org/10.3390/fermentation5010009

Palaniraj A., and Jayaraman V. (2011) Production, recovery and applications of xanthan gum by Xanthomonas campestris. Journal of Food Engineering 106(1): 1-12. Available at: https://doi.org/10.1016/j.jfoodeng.2011.03.035

Purwadi R., Rizki Z., and Aprianti F. P. (2009) Cabbage Extract as a Precursor in Xanthan Gum Production using Xanthomonas Campestris. 16th Asean Regional Symposium on Chemical Engineering 402-404.

Rončević Z. et al. (2017) Xanthan production on wastewaters from wine industry. Hemijska Industrija 71(2): 145-153. Available at: https://doi.org/10.2298/HEMIND160401025R

Salah R. B., Chaari K., Besbes S., Blecker C., and Attia H. (2011) Production of xanthan gum from xanthomonas campestris nrrl b-1459 by fermentation of date juice palm by-products (phoenix dactylifera l.). Journal of Food Process Engineering 34(2): 457-474. Available at: https://doi.org/10.1111/j.1745-4530.2009.00369.x

Sirohi S., and Kumar A. (2019) Batch Production Kinetics of Xanthan Gum From Potato Waste. Think India Journal 22(17): 66-72.

Soleymanpour Z., Nikzad M., Talebnia F., and Niknezhad V. (2018) Xanthan gum production from acid hydrolyzed broomcorn stem as a sole carbon source by Xanthomonas campestris. 3 Biotech 8(7). Available at: https://doi.org/10.1007/s13205-018-1322-z

InfraRed Absorption Spectra Table. Available at: https://chem.libretexts.org/Ancillary_Materials/Reference/Reference_Tables/Spectroscopic_Reference_Tables/Infrared_Spectroscopy_Absorption_Table (accessed on 02 April 2024).