ANNALS OF MULTIDISCIPLINARY RESEARCH, INNOVATION AND TECHNOLOGY (AMRIT)
(A peer-reviewed open access multidisciplinary journal)
www.adtu.in/amrit

RESEARCH ARTICLE

Production of Xanthan gum using Food and Beverage waste and potential use of Xanthan as an agar substitute

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Article Chronicle: Received: 30.06.24 Accepted: 30.11.24 Published: 31.12.24

Abstract

Xanthan gum, a versatile biopolymer derived from Xanthomonas campestris via fermentation, has garnered significant attention for its commercial applications in various industries, particularly in food and cosmetics. However, its production cost remains high due to the expensive carbon sources utilized in fermentation. This study explores the feasibility of utilizing food and beverage waste, including watermelon peels, banana peels, bakery waste, and rice starch water, as economical and eco-friendly alternatives for xanthan fermentation. X. campestris NCIM 2961 was employed for fermentation, and various parameters such as pH, temperature, incubation period, and agitation were optimized to enhance xanthan yield. Results indicate that alternative substrates exhibit potential for xanthan production, with certain conditions yielding comparable or even higher gum yields compared to standard media. Another objective of this study was to see the potentiality of xanthan gum as an agar substitute. Growth of microorganisms such as E. coli, S. aureus and S. cerevisiae was successful in xanthan-substituted agar plates. This research underscores the prospect of valorising waste streams for sustainable biopolymer production, offering both economic and environmental benefits.

Keywords: *Xanthan gum, food and beverage, watermelon, banana, bakery, rice water, agar substitute*

List of abbreviations used: WWP - Waste Watermelon Peel, BP - Banana Peel, BW - Bakery Waste, RW- Rice Water

1 Introduction

Xanthan gum is a biopolymer obtained from plant pathogen, *Xanthomonas campestris* via fermentation. Xanthan gum was the second exopolysaccharide to be commercially produced, following dextran. Excellent solubility, heat resistance, pH stability, high viscosity, pseudo-plasticity, and crosslinking are some of the qualities that make xanthan gum a great option for a variety of commercial applications, particularly in the food and cosmetics sectors. Xanthan gum stands out from other natural biopolymers due to its unique capacity to function in organic solvents, acids, bases, and even saltwater conditions without causing viscosity changes. D-glucose repeating units make up the primary chain of xanthan gum, while mannose and glucuronic acid make up the two side chains. Alpha-1,2 glycosidic linkages and the other side chain bind the mannose side chains to the main chain. A series of sequential steps are involved in the industrial

fermentation of xanthan. First-stage commercial xanthan manufacturing entails fermenting *Xanthomonas sp.* on an appropriate medium with a carbon source. (usually glucose) and enhanced under ideal reaction conditions with nitrogen, salts, and trace elements. However, the industrial carbon source utilized in xanthan fermentation is quite expensive, which drives up manufacturing costs and raises the price of the finished product. This necessitates an economical substitute. One such potential substrate that has garnered attention is food and fruit waste. The main objective of this study was to evaluate the ability of fruit and food waste (bakery waste, rice water, banana peels, and watermelon peels) to serve as an economical, eco-friendly carbon source for xanthan fermentation.

In an earlier study (1) it was found that waste bread hydrolysate was used as a bio-source for xanthan production by various isolates and standard bacteria and the highest gum yield was 14.3 g/l. Bakery waste was also a good source of organic substrate that can be electron donors (2). In another study, it was found that bakery waste, including cakes and pastries from Starbucks Hong Kong, was evaluated for the potential of succinic acid production (3). A study studied that bakery waste biogas was produced using a semi-continuous, two-stage anaerobic digestion system, consisting of 2 l-first stage digesters and 5 l-second stage digesters under the temperature of 30ºC (4).

Banana peels are typically dumped into the environment without any treatment. In some cases, banana peel may be used as organic fertilizer because of its high fibre content (5). Approximately 36 million tons of banana peel are produced every year, and their current endpoint is associated with adverse environmental impact and economic losses (6). The global production of bananas is 116 million tonnes during 2019, and the banana fruits are obtained throughout the year. The fruit average is 125 grams, of which approximately 75% is water and 25% dry matter content. Cellulose content, Hemicellulose content, Pectin content, and low cost and availability are some of the qualities of banana peels that may make them a viable source for the synthesis of xanthan gum.

Rice is one of the world's most abundant cereal crops with production concentrated in countries like China, India, Indonesia, Bangladesh, and Vietnam. Annual global rice production exceeds 700 million metric tons, with Asia accounting for the majority of production, generating vast quantities of starch wastewater during its processing. This byproduct, often considered a waste stream, presents a promising opportunity as an alternative substrate for xanthan gum production. Utilizing rice starch water not only adds value to this otherwise discarded material but also contributes to the sustainable utilization of agricultural resources. One of the studies (7) done with Hydrolysed Rice Bran (HRB) fermentation resulted in xanthan production that was higher than sucrose fermentation for all isolates. The results show that the highest yield of xanthan gum among the two strains was obtained using the HRB media by *X. campestris* NRRL B-1459 (21.87±1.144 g/L), followed by *Xanthomonas campestris pv. campestris* (17.1±0.565 g/L gum). The high yield of HRB may be due to its [sugar composition which includes a reducing sugar (glucose).

According to a recent study published (8), out of the overall mass of a watermelon, the rind part contributes 30% and contains a decent amount of fibre, carbohydrates and wax. Additionally, watermelon peel waste can be used as a growing medium for bacteria that produce cellulose. From the leftover waste watermelon peels, microorganisms can make biopolymers, and utilizing these organic wastes encourages the development of a circular bio-economy.

Another objective of this study was to see the potentiality of xanthan gum as an agar substitute. A study showed that on a media which is solidified with xanthan (0.7%) in combination with agar (0.3%) growth of fungi and bacteria was better than normal agar plate (9). Xanthan gum is used as a thickening agent in the food industry. To utilise this property, xanthan gum was used to create microbiological plates at a lower cost than agar. The present study showed that microorganism growth was satisfactorily supported by the xanthan media. Therefore, in microbiological agar medium, the combination of xanthan gum and agar can be utilized as an inexpensive gelling agent in place of expending agar.

Table 1: List of some previously experimented substrates

Substrate	Time	Temperature	pH	Xanthomonas strain used	Yield (in g/L)	Reference
Carob extract	54hr	30ºC	7.0	*X.Campestris* NRRL B-1459	11.5	(10)
Sugar beet molasses	120hr	28ºC	7.2	Wild-type strain of *X. campestris* NRRL B-1459	19.8	(11)
Pineapple peels	72hr	30ºC	7.0	*X. campestris* ATCC	8.48	(12)
Jackfruit seed powder	72hr	32ºC	7.0	*X. campestris* NCIM 2961	51	(13)
Potato waste	96hr	28ºC	7.0	*X. campestris* NCIM 2961	45	(14)

2 Materials and Methods

2.1 Bacterial strain and media

For all of the tests in this investigation, a pure bacterial culture of *X. campestris* NCIM 2961 was obtained from the National Collection of Industrial Microorganisms (NCIM), Pune. The culture media utilized were MGYP medium (0.3 g/l of malt extract, 1 g/l of glucose, 0.3 g/l of yeast extract, and 0.5 g/l of peptone in 100 ml of distilled water). The bacterial culture was consistently maintained throughout the studies by periodically subculturing it in MGYP agar plates (2% agar-agar) to prevent culture degradation.

2.2 Food and Beverage waste utilized

Watermelon peels (150g), Banana peels (50g), Bakery waste (50g) and Rice starch water (100 ml) were used to prepare the alternative medium each round of the experiment.

2.3 Liquid media culture

2.3.1: Standard MGYP media:

To serve as the standard media, 300 millilitres of MGYP liquid medium were prepared in distilled water. With the use of 1N HCl and 1M NaOH solutions, the pH of the produced medium was brought to 6.8. After dividing the medium equally into three portions (100 ml each), it was introduced to three Erlenmeyer flasks and autoclaved before being inoculated and fermented.

2.3.2 Alternative media:

For this study, the alternative media using food and beverage waste (excluding the Rice starch water) was prepared by involving various procedures starting from grinding the substrate after the addition of distilled water, filtering the obtained paste using a muslin cloth, obtaining only its liquid content. Distilled water was added to dilute and make up a total volume of 300 ml each for each type of waste which was then divided into 100 ml each in three Erlenmeyer flasks. Moreover, the rice water media was prepared by taking 100 ml of rice water in a 500 ml beaker and 200 ml of distilled water was added to lessen the viscosity making up a total volume of 300 ml which was then divided into 100 ml each in three Erlenmeyer flask. The pH of the media was adjusted to 6.8 using 1N HCl and 1M NaOH solutions which was then autoclaved at 121oC and 15 psi for 20 mins before inoculation. For experiments in which the pH had to be varied, the media was adjusted to a suitable pH each time.

2.4 Fermentation

The initiation of the fermentation was carried out by introducing a loopful of culture into the sterile standard and alternative media. 250 ml Erlenmeyer flasks were used throughout the process for their headspace to provide optimum gaseous exchange during the fermentation which was then incubated in a shaking incubator (SCIGENICS ORBITEK).

2.5 Variation of Fermentation Parameters

To study the effects of pH, temperature, incubation period, and agitation on the yields of xanthan gum, these parameters were diversified at different levels and optimized in different experiments for each parameter (pH 6, 6.5, and 6.8, Temperature 28oC, 30oC, and 32oC, Incubation 48 hrs, and 72 hrs, except for rice water media in which 24 hrs duration was also included, Agitation 100, 150, and 200 rpm) to determine the ideal and best fermentation conditions for the production of xanthan. The first test was of the effects of pH in which the alternative substrate media were inoculated and incubated at a constant temperature of 30oC, 100 rpm agitation for 24 hrs for rice water substrate and 48 hrs for other substrate media with varying pH adjusted to 6, 6.5, and 6.8, respectively with each experiment before inoculation. Based on the highest xanthan yield, the best pH was selected. Next, the temperature for fermentation of the rice water media was varied at three levels, 28oC, 30oC, and 32oC keeping the pH the same in which the yield was the highest. The duration of the incubation period was varied where the durations were 48 hrs and 72 hrs (24 hrs, 48 hrs and 72 hrs for RWM) and the parameter with the highest yield was considered. Lastly, the agitation was set as a parameter varying from 100 rpm, 150 rpm, and 200 rpm and the parameter with the highest yield was considered.

2.6 Xanthan gum precipitation

On completion of the fermentation, xanthan gum was precipitated using isopropanol. Double the volume of isopropanol was added to the fermentation broth and chilled in the refrigerator for 1 hr after which it was centrifuged using a cooling centrifuge at 10000 rpm, 4oC for 10 mins to separate the biopolymer. The supernatant was discarded and the pellet was extracted and dried in the hot air oven at 80oC for 2 hrs. The dried xanthan was weighed using an electronic balance and the weight was recorded.

2.7 Xanthan gum as Agar substitute

An attempt was made to test the extracted Xanthan as a microbial gelling agent and to substitute agar which is commonly used in microbiological work. The commercially available Xanthan was used for this purpose in combination with commercially available agar procured from HiMedia® India unless otherwise indicated. The combinations that were tested were Xanthan + agar (1%+0.5%) and Xanthan + agar (1.5%+0.5%) for the commercially available Xanthan (Tables 2, 3 and 4). The media used to test the gelling were Nutrient broth and fungal broth acquired from HiMedia® India unless otherwise indicated. Control agar plates of these three media were also prepared. These media were sterilized and poured into pre-sterilized Petri plates in a Laminar Air Flow unit BSL-1 (Esco®). Organisms such as Escherichia coli, Staphylococcus aureus, and Saccharomyces cerevisiae were obtained from the Microbiology Department of Assam down town University and were streaked on the prepared

media. After an incubation period of 24 hours, the growth was observed. These experiments were performed in repetitions.

3 Results

3.1 Observation of parameters

3.1.1 *pH variation*

Experiments with different substrate media and *X. campestris* revealed that the highest yield in Standard media (MGYP) at 30ºC, 100 RPM agitation to be at a pH = 6.8. In the case of WWP media, the highest yield was at a pH of 6.8 (6.6 g/L) {fig 1}. In the case of RW media, the highest yield was at a pH of 6.5(3.33 g/L) {fig 5}and in BW media, the highest yield was at a pH of 6.5(11.15 g/L) {fig 1} and lastly, in case of WBP media, the maximum yield was at a pH of 6.5 (10.5 g/L).

3.1.2 *Variation of incubation period*

The duration of incubation for standard MGYP media and other alternative media were varied at different level of 48 hrs and 72 hrs (except RW media i.e. 24 hrs, 48 hrs and 72 hrs) to experiment with the incubation period for the highest yield of xanthan gum. It was noticed that in case of standard MGYP media, the duration with the highest yield was at the duration of 48hrs. In the case of the other alternative media (WWM, WBP and BW) the maximum yield of xanthan gum was observed at 48 hrs (WWP media, 6.6 g/L; BW media, 11.5 g/L; WBP media, 10.5 g/L {fig 2} whereas the RW media gave a maximum yield result at 72 hrs 4.13 g/L {fig 7}.

3.1.3 *Temperature variation*

The temperature for the incubation of standard MGYP media and alternative media were varied at the levels of 28ºC, 30ºC and 32ºC to achieve the highest yield of xanthan gum. It was observed that at 30ºC C the the production of xanthan was highest in the case of standard MGYP media. Whereas in the case of WWP media, WBP media and BW media, it was observed that the highest yield was at the temperature of 30ºC (WWP media 6.6 g/L; WBP media 10.5 g/L; BW media 11.5 g/L) {fig 3} and Rice water media gave the maximum yield at 28ºC (4.13 g/L) {fig 6}.

3.1.4 *Variation of agitation*

The agitation for the incubation of standard MGYP media and alternative media was varied at the levels of 100 rpm and 150 rpm to experiment with the highest yield of xanthan gum. It was observed that at 100 rpm production of xanthan was highest in the case of standard MGYP media. Where in the case of WBP, WWP, and BW media at 100 rpm it was observed highest yield i.e. WBP media 10.5 g/L; BW11.5 g/L; WWP 6.6 g/L {fig 4} and rice water media gave maximum yield at 100 rpm (4.13g/L) {fig 8}.

3.1.5 *FTIR analysis of xanthan gum*

The FTIR spectra of the Xanthan gum obtained from Standard MGYP media (fig 14) and wastes were compared with that of food-grade Xanthan gum to validate the results. Spectral data from FTIR of commercial food-grade xanthan (fig 13) shows close similarity to that for xanthan extracted from the waste media (Fig 9,10,11,12). As a result, xanthan from wastes and standard commercial xanthan gum have almost identical spectral characteristics that validate their identity.

3.2 Utilization of xanthan gum as a potential agar substitute for microbiological plates

The solidification property of commercially available Xanthan was tested. Xanthan gum alone (commercial) failed to replace agar in the Nutrient Broth medium. However, efficient solidification was achieved in media containing 1% HiMedia Xanthan + 1% agar and 1.5% HiMedia Xanthan + 0.5% agar. These combinations were further employed for the preparation of Nutrient Agar and Fungal Agar medium, followed by isolation of *Staphylococcus aureus*, *Escherichia coli* and *Saccharomyces cerevisiae*. 0.5% HiMedia xanthan + 1.5% agar was used to culture *S. aureus* and *E. coli*, also for *E. coli* a combination of 1% HiMedia xanthan + 1% agar was used. 1% HiMedia xanthan + 1 % agar along with dextrose, peptone and yeast extract was used for culturing *S. cerevisiae.* All three of the microorganisms were successfully cultured in plates in combination with xanthan, although the growth of *S. cerevisiae* was minimally less (Fig 15,16,17,18). The novel media supported the growth of three different strains of micro-organisms, (*Escherichia coli*, *Staphylococcus aureus* and *Saccharomyces cerevisiae*). The novel media is eco-friendly, biocompatible, soluble and has a stable nature.

4 Discussion

As industries grow, more waste is produced, which makes waste management more difficult and creates environmental problems. However, these problems may be resolved by using industrial waste as a substitute source of fermentation, which will also lower production costs and, as a result, lower the price of the final product.

The findings of the present study indicate that *X. campestris* NCIM 2961 can generate xanthan gum utilizing WWP, RW, BP and BW from the food and beverage industries.

In comparison to traditional MGYP media, the alternate-based medium utilized in this investigation produced more xanthan gum during standard circumstances of the reaction. An earlier study (15) showed the production of xanthan gum by fermenting spent grain media, a waste of the beverage industry.

The FTIR analysis of xanthan derived from WWP, RW, BP and BW based medium and food-grade xanthan yielded remarkably similar spectrum data which attributes and verifies the purity of xanthan made using alternate-based media. By the variations of parameters (pH, temperature, duration and agitation) and setting them at different levels over time a significant improvement in the final product yields were achieved.

From the results and findings of these experiments with alternate waste-based mediums, it was confirmed that the alternate mediums used for this study can also be used as an alternative

source for the production of xanthan gum after further studies based on the toxicity of the xanthan gum produced by these specific waste-based mediums needs to be done. An earlier study (16) has shown that food and beverage waste can be a viable substrate for successful xanthan gum production via fermentation.
Another experiment was done along with the production of xanthan gum from alternate waste-based mediums. In this study, pure xanthan gum from HiMedia was utilised to replace agar in microbial plates. It was found that xanthan gum alone could not replace agar. In yet another experiment, Xanthan gum and Agar were both combined at a concentration of 1% each. The results were significantly improved. Lastly, the experiment was also conducted to grow microorganisms in these plates. Here the same concentration of Xanthan gum and Agar was taken and 0.65% of Nutrient Broth was added to provide nutrition to the microorganisms in order to grow them. Here, a gram-positive bacterium (*Staphylococcus aureus*) and gram-negative bacteria (*E. coli*) were inoculated and cultured successfully. This study has revealed the potential of Xanthan gum to replace Agar in microbiological plates, however, further studies in this field need to be done in the future to maximize the results of replacing Agar with xanthan gum.

Conclusion

This study advances the environmental concerns regarding the wastes from food and beverage industries and constructively addresses the problems regarding the management of the waste substrates used in these experiments. The study underlines the understanding of the commercial value of some of the wastes from the food and beverage industries. This study has approached a sustainable and recyclable way of producing xanthan gum by utilization of waste-based media from food and beverage industries which can significantly reduce the cost of production of xanthan gum.

Acknowledgement

The authors are grateful to the Department of Biotechnology, Faculty of Science, Assam down town University for their valuable support throughout the study. The authors also acknowledge with thanks the help received from Dr. Sangeeta Singha, Department of Microbiology, Assam down town University for providing the *Staphylococcus aureus* culture.

Conflict of interest

The authors have no conflicts of interest to declare.

Author's contribution

BPC and SL conceived the idea, JS, DR, LL and TDB executed the project and wrote the manuscript.

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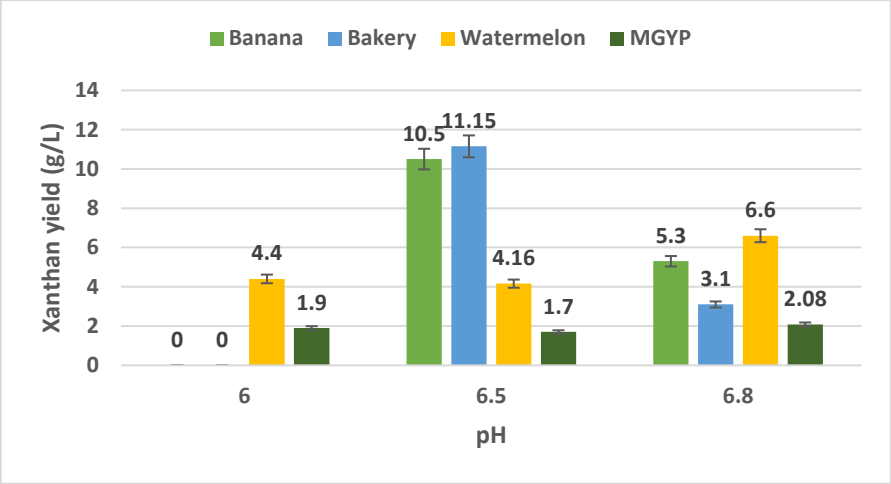

Figure 1: Comparison of xanthan yield from waste media and standard MGYP media at different pH (6.0, 6.5, and 6.8) at 30°C, 100 rpm agitation, and 48 hrs. of incubation.

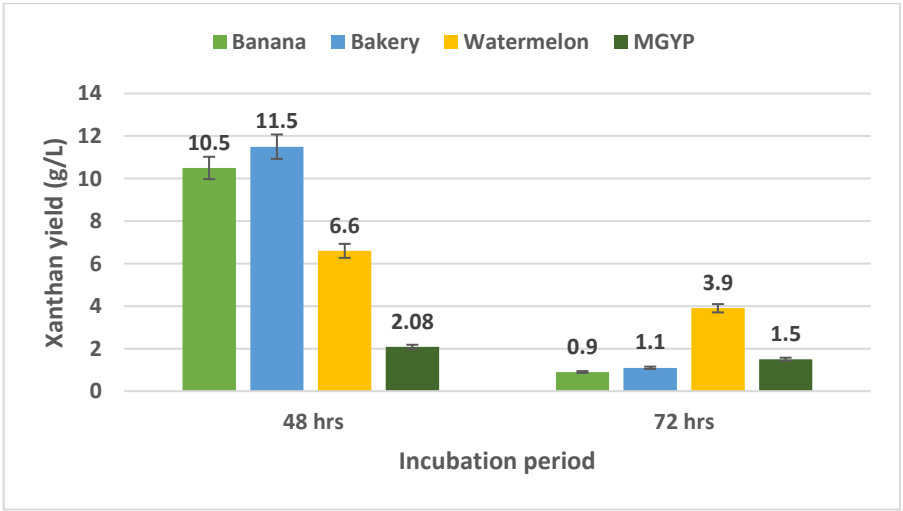

Figure 2: Comparison of xanthan yield from waste media and standard MGYP media at different incubation period (48 hrs. and 72 hrs.) at 30°C, pH 6.5 (Banana and bakery waste media), 6.8 (Watermelon waste media) and 100 rpm agitation.

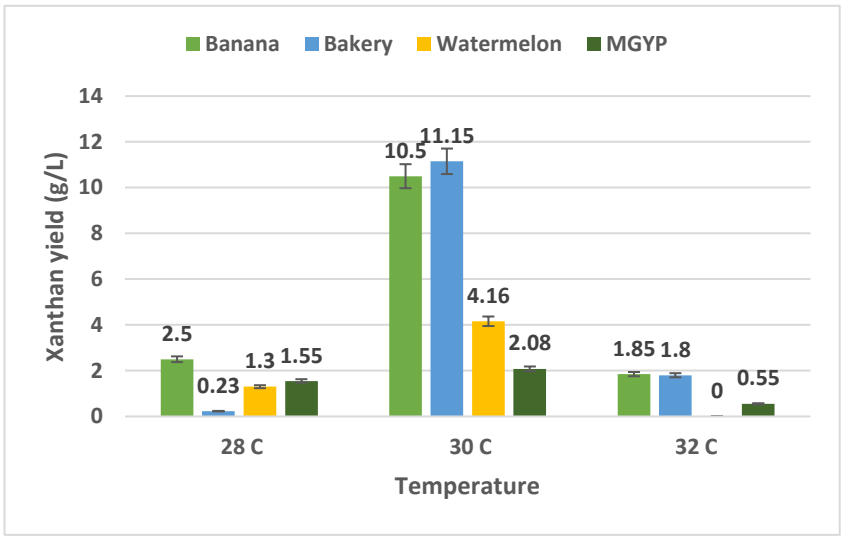

Figure 3: Comparison of xanthan yield from waste media and standard MGYP media at different temperatures (28ºC, 30ºC, and 32ºC), pH 6.5 (Banana and bakery waste media), 6.8 (Watermelon waste media), 100 rpm agitation, and 48 hrs. of incubation.

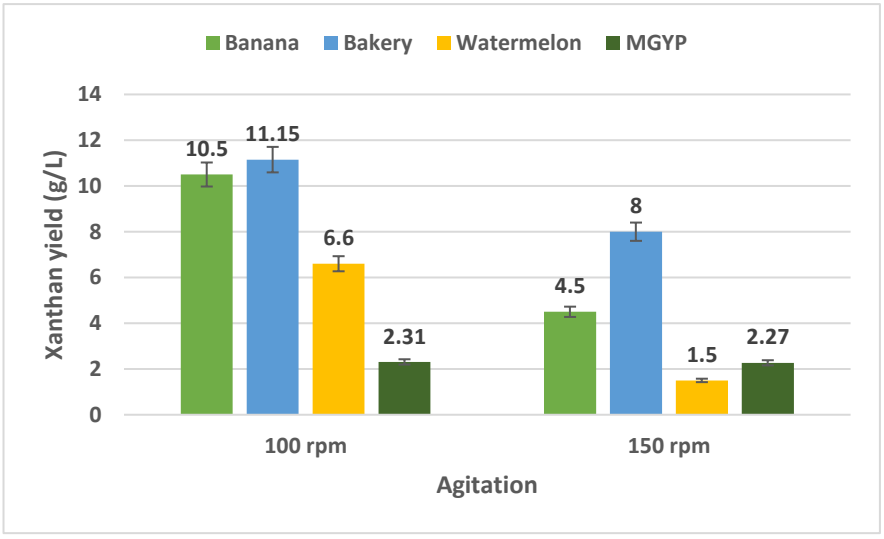

Figure 4: Comparison of xanthan yield from waste media and standard MGYP media at different agitation (100 and 150 rpm), pH 6.5 (Banana and bakery waste media), 6.8 (Watermelon waste media), 30 ºC, and 48 hrs. of incubation.

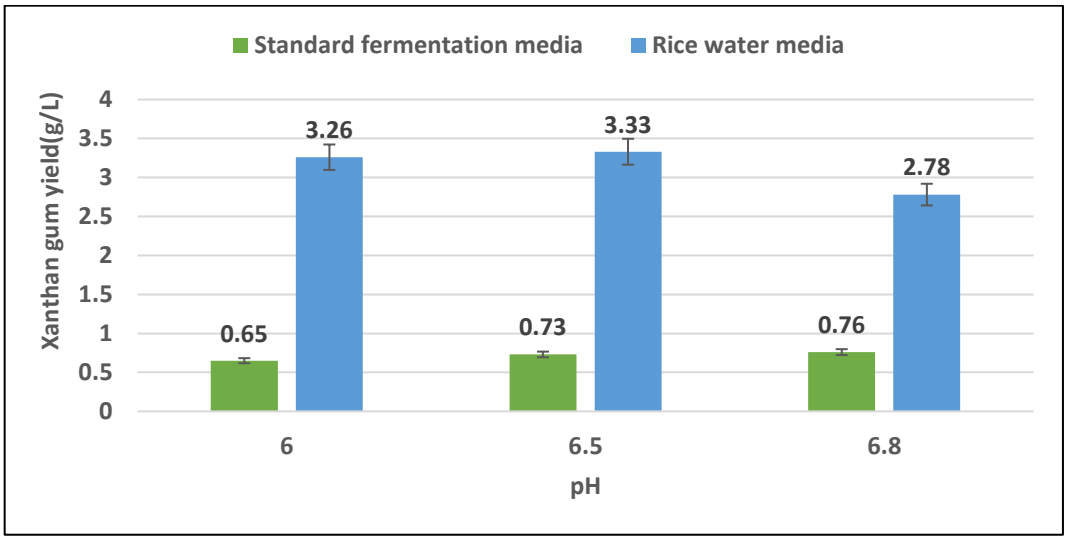

Figure 5: Comparison of xanthan yield from standard MGYP media and Rice starch water media at different pH (6.0, 6.5, and 6.8) at 30℃, 100 rpm agitation, and 24 hrs of incubation.

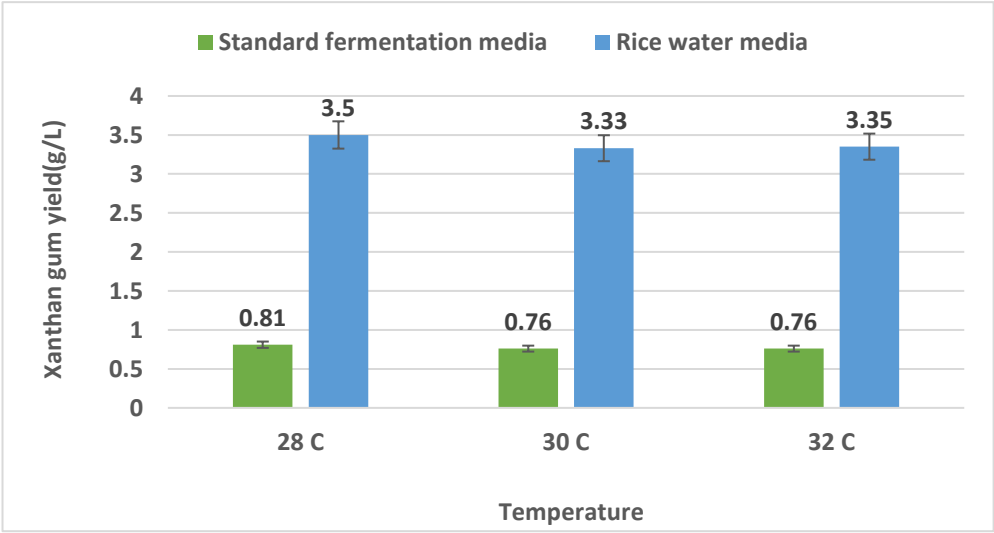

Figure 6: Comparison of xanthan yield from standard MGYP media (pH 6.8) and Rice water media (pH 6.5) at different temperatures (28℃, 30℃, and 32℃) at 100 rpm agitation, and 24 hrs. of incubation.

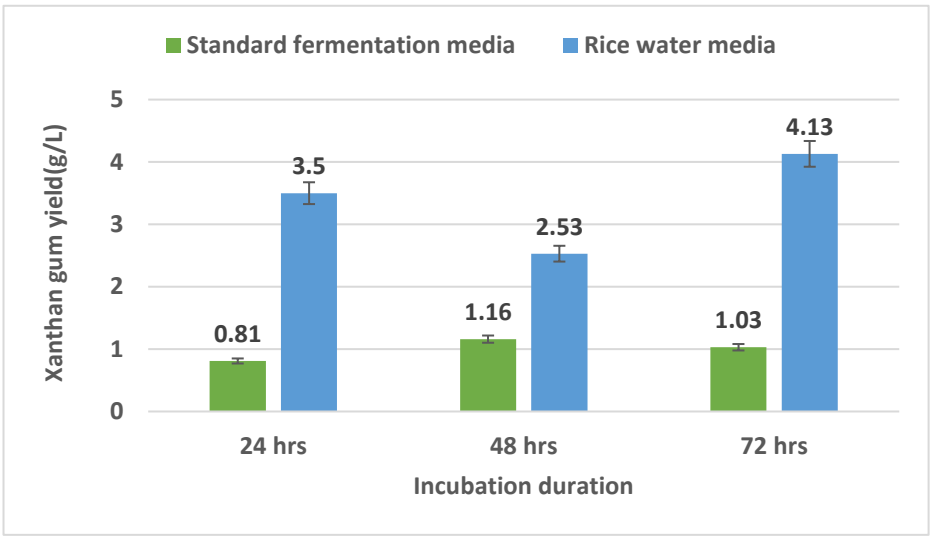

Figure 7: Comparison of xanthan yield from standard MGYP media (pH 6.8) and Rice water media (pH 6.5) at different incubations (24 hrs, 48 hrs, and 72 hrs) at 28℃, and 100 rpm agitation.

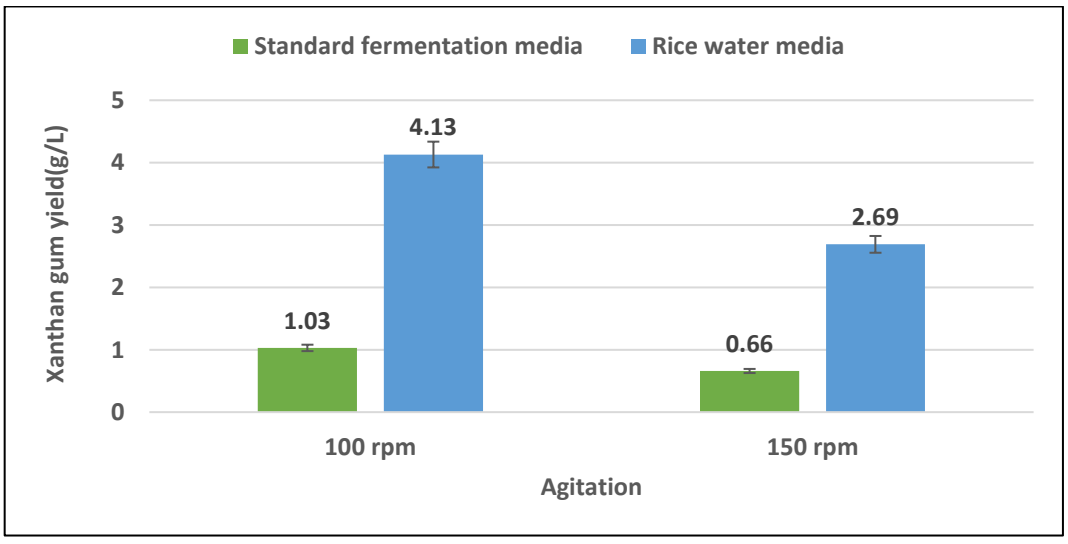

Figure 8: Comparison of xanthan yield from standard MGYP media (pH 6.8) and Rice water media (pH 6.5) at different agitations (100 and 150 rpm) at 28℃, and 72 hrs. of incubation.

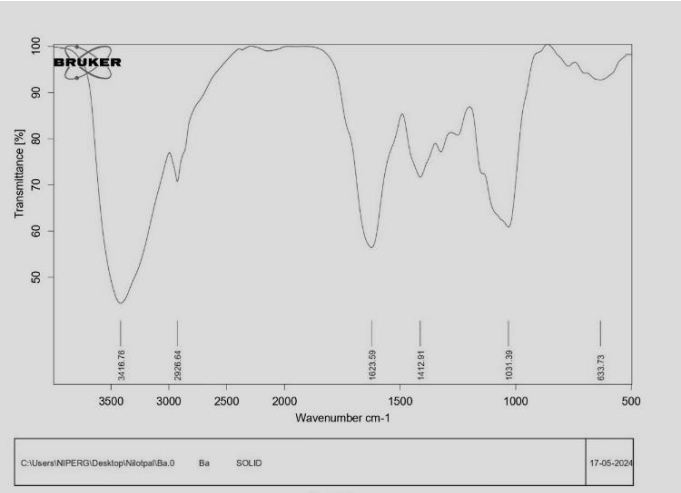

Figure 9: FTIR spectra of xanthan gum from the fermentation of Banana peel media

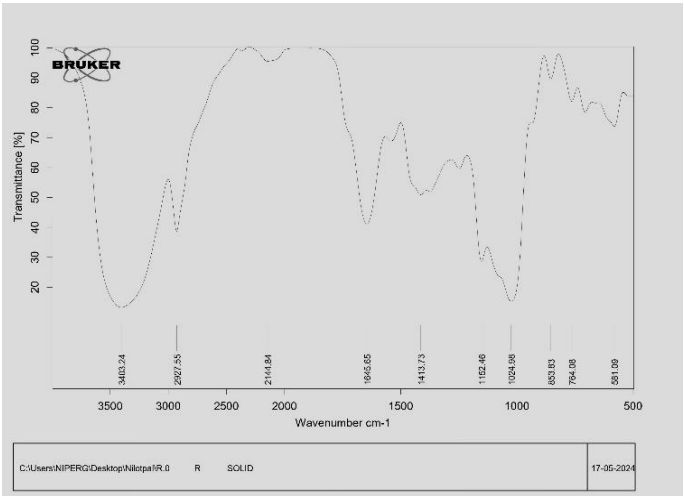

Figure 10: FTIR spectra of xanthan gum from the fermentation of Rice water media

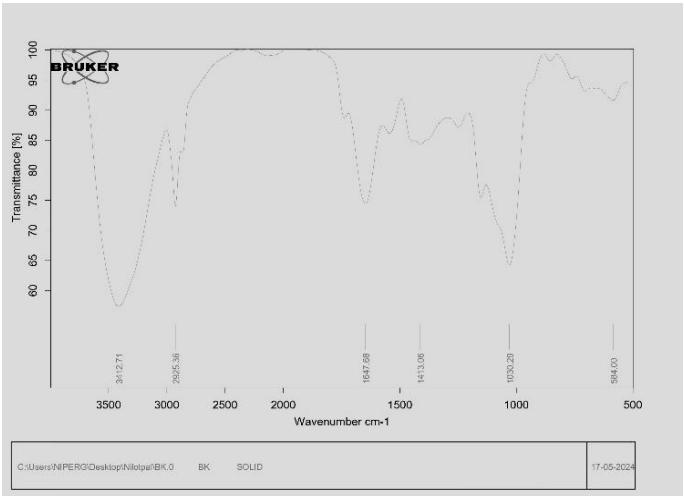

Figure 11: FTIR spectra of xanthan gum from the fermentation of Bakery waste

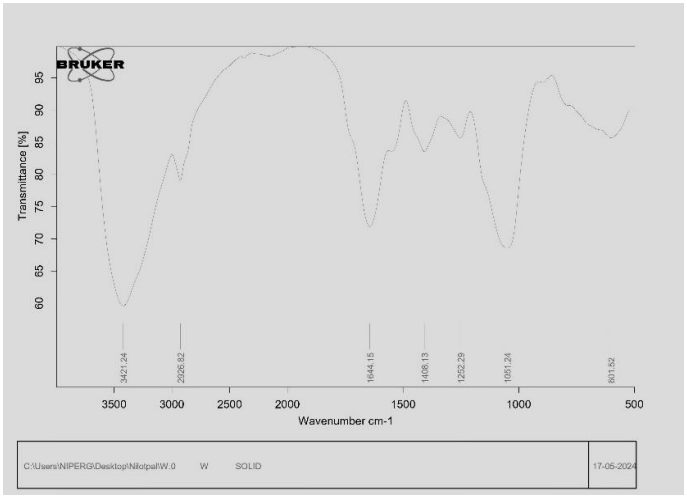

Figure 12: FTIR spectra of xanthan gum from the fermentation of Watermelon peel

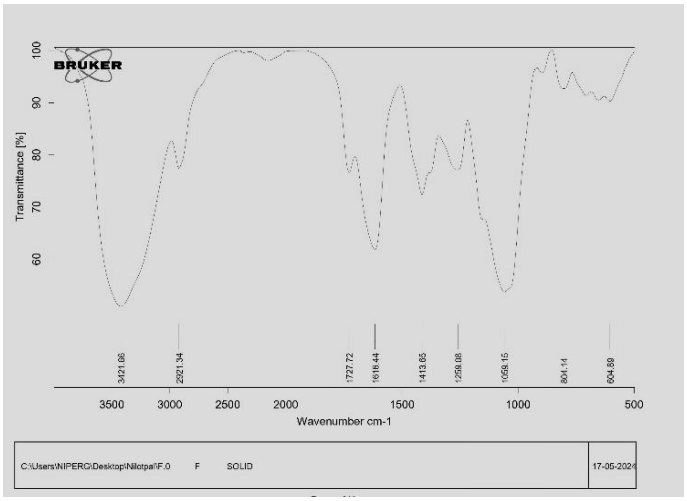

Figure 13: FTIR spectra of Food grade xanthan gum

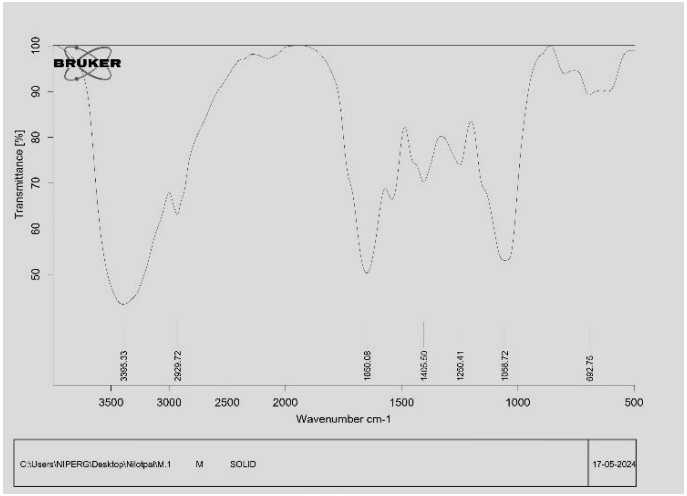

Figure 14: FTIR spectra of xanthan gum from the fermentation of Standard MGYP media

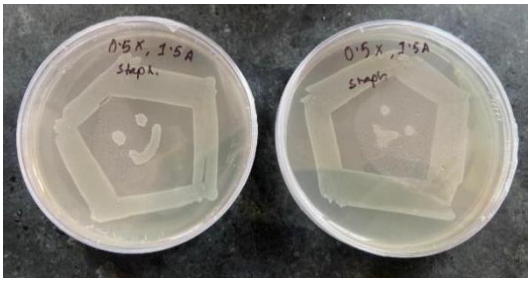

Figure 15: *S. aureus* growth (0.5% xanthan + 1.5% agar + Nutrient broth)

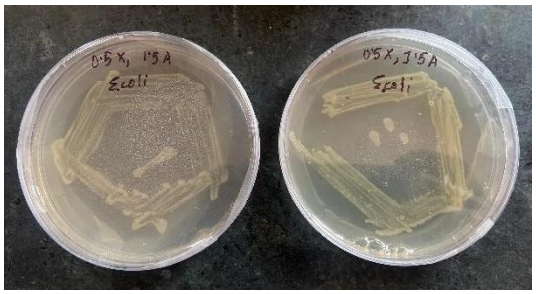

Figure 16: *E. coli* growth (0.5% Xanthan + 1.5% Agar + Nutrient Broth)

Figure 17: *E. coli* growth (1% Xanthan + 1% Agar + Nutrient Broth)

Figure 18: *S. cerevisiae* growth (1% Xanthan + 1% Agar + Dextrose + peptone + yeast extract)

Table 2. Xanthan as agar substitute in growing *Staph aureus*

Bacterial Strain: *Staphylococcus aureus*	
Percentage of Gelling Agent Used (%)	**Solidification**
0.5% xanthan + 1.5% agar + 0.65% Nutrient Broth	**Yes**
1% xanthan + 1% agar	**Yes**
1% xanthan + 1% agar + 0.65% Nutrient Broth	**Yes**
0.75% xanthan + 0.25% agar	**No**

Table 3. Xanthan as agar substitute in growing *E.coli*

Bacterial Strain: *E. coli*	
Percentage of Gelling Agent Used (%)	**Solidification**
1% xanthan + 1% agar	**Yes**
1% xanthan + 1% agar + 0.65% Nutrient Broth	**Yes**
0.5% xanthan + 1.5% agar + 0.65% Nutrient Broth	**Yes**
0.75% xanthan + 0.25% agar	**No**

Table 4. Xanthan as agar substitute in growing *S. cerevisiae*

Fungal Strain: *Saccharomyces cerevisiae*	
Percentage of Gelling Agent Used (%)	**Solidification**
1% xanthan + 1% agar	**Yes**
1% xanthan + 1% agar + Dextrose + Peptone + Yeast extract	**Yes**