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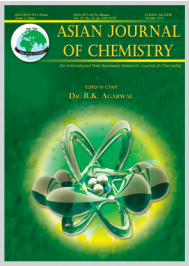

Production and Characterization of Xanthan Gum with *Xanthomonas campestris* from Palm Syrup

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In this work, the effects of reaction temperature on xanthan gum production were investigated. Xanthan gum was produced in batch fermentation by *Xanthomonas campestris* PTCC 1473 from palm syrup. Fermentations were carried out at different temperatures (20-40 ºC) and 500 rpm with neutral pH. In addition, during experiments, broth viscosities were measured. The results specified that maximum xanthan gum productions were obtained 15.5 g/L from palm syrup at 30 ºC. Furthermore, the chemical structures of the obtained products were evaluated by FTIR spectroscopy.

Key Words: Xanthan gum, *Xanthomonas campestris*, Palm syrup, Temperature, Broth viscosity.

INTRODUCTION

Xanthan gum is a microbial polysaccharide that can obtain based on waste materials. It is produced by a pure culture fermentation of a carbohydrate with naturally occurring bacterium *Xanthomonas campestris*[1-3]. Xanthan gum has specific properties that make it extremely practical (pseudoplastic rheological flow, improves product moisture, rheology control agent in aqueous systems, neutral flavour, effective viscosity builder, prevents fat separation, stabilizes emulsions and suspensions, pH stable over a broad period[4,5]. In addition to many uses in food products (mayonnaise and salad dressing formulations, in bakery fillings, cake mixes, in ice cream, in squeezable chewing gum, in creamed cottage cheese, in yogurt milk shakes, in instant soups). It has found numerous applications in cosmetics and pharmaceuticals (in toothpastes, in liquid pharmaceutical products, in cosmetics and toiletries, in barium sulfate solutions for use as radiopaque media) as thickening agents, in coatings, films, hydrogels, microspheres, nanoparticles and matrix tablets[6-9].

Commercially existing xanthan gum is comparatively expensive because of glucose and sucrose often used as the carbon source. The rate of fermentation medium signified a critical aspect of the commercial production of xanthan gum[1,4,10].

The use of cheaper substrate, instead of the customary used glucose or sucrose, might result in a lower cost of the final product. For cost reduction could be acceded to low cost substrates, such as waste agricultural products (palm syrup, molasses, waste starch)[11,12]. The purpose of present research paper is to study batch fermentation for xanthan gum production from low cost substrates like palm syrup. In addition, optimal operating temperature, viscosity of fermentations broth and FTIR spectroscopy were suitable indicators to monitor the production rate and the structure of xanthan gum.

EXPERIMENTAL

Microorganism and inoculum preparation: *Xanthomonas campestris* PTCC 1473 was supplied by Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. The microorganism was grown on agar medium containing (g/L): yeast extract 5.0, peptone 5.0, glucose 10.0 and agar 20.0 (Merck, Germany). The propagated cells for the inoculums were grown in a medium (the stated compositions excluding agar) at neutral pH in a 250 mL flask with 100 mL of culture medium in an incubator shaker (Stuart, S1500 series, USA) for 24 h. The incubator shaker was set at 200 rpm and 28 ºC to enhance oxygen mass transfer rate into the media. Thus, maximum cell growth was obtained and the revived fresh inoculum of the bacteria was used for each experimental run. The stock culture stored on slant agar was maintained at 4 ºC and the subcultures were revived for every 2 weeks to avoid strain degradation.

Preparation of palm syrup: Initially the concentrated palm syrup were diluted with distilled water (300 g commercially palm syrup were diluted to 1 L). Dilute acid hydrolysis

was carried out to enhance the dextrin and monomeric carbohydrates of palm syrup. Concentrated HCl (2.5 mL) added to 1 L of the diluted syrup. The pretreatment was prolonged for 24 h. The pretreated syrup were autoclaved at 121 ºC for 15 min, after that neutralized with dilute NaOH and the reduced sugar was measured using DNS in colorimetric method.

Xanthan gum production: The production of xanthan gum from palm syrup was carried out in 1000 mL Erlenmeyer flasks with 300 mL of medium. It contains (g/L): glucose, sucrose, fructose 30, yeast extract 5 and additive solution 3 mL (consist of KH_2PO_4 (3 g/L), $MgCl_2$ (0.6 g/L), Na_2SO_4 (0.1 g/L), H_3BO_3 (0.006 g/L), ZnO (0.006 g/L), $FeCl_{36}H_2O$ (0.02 g/L), $CaCO_3$ (0.02 g/L)), that glucose was obtained from hydrolyzed and pretreated palm syrup.

Batch experiments were conduct for xanthan gum production with variation of temperature in the range of 20-40 ºC. The fermentations were commenced with inoculums size of 7 % (v/v), experiments were conducted at stirrer rate 500 rpm on a magnetic hot-plat stirrer (VELP, Italy). Runs were terminated after 72 h of incubation. The pH was initially neutral and was not controlled by any titrants throughout the runs. All experimental runs were replicated and averaged values were reported in this work.

Analytical methods: During fermentation, for evaluating the xanthan gum, it was recovered by centrifuging at (10000 g) for 0.5 h at 4 ºC in order to sediment the cells. Xanthan gum in the supernatant was precipitated using ethanol, methanol or acetone (1:3 v/v) (Fluka, Germany). The solution was maintained at 4 ºC for 24 h and re-centrifuged at 10000 g, for 0.5 h at 4 ºC. The precipitate was diluted in distilled water and dried at 50 ºC in a conventional oven (Binder, Germany) until constant weight, to determine the xanthan gum content. Cell dry weigh was also determined using a cellulosic filter, 25 mm diameter and 0.25 pore sizes (Wathman, USA) then the filter were dried in an oven at 80 ºC for 24 h and weighed. The medium viscosity was measured by Siemens glassy viscometer. FT-IR spectra were recorded on the dried powder of xanthan was analyzed with Fourier transform infrared (FTIR, Bruker, Model Vector 22, Germany) to define the functional group of synthesized xanthan gum. The dry sample powder was mixed with KBr and pressed into pellets under reduced pressure. The FTIR spectra were obtained by scanning between 4000 and 500 cm^{-1}.

RESULTS AND DISCUSSION

In this study, xanthan gum production of palm syrup were discussed. The yield of xanthan gum and the mass of cell dry weight were obtained. Fig. 1 shows the maximum amount of produced xanthan gum 15.5 g/L. It was reported in the literatures that monosaccharide was identified as the suitable carbon source for xanthan gum production[1,3,5]. Since the treated palm syrup enriched with inverted monomer of sugars, the yield of xanthan production was increased. The palm syrup showed fair results because of it might have natural polymeric sugars like starch that could affect on xanthan gum production. In addition, in this figure the mass of cell dry weight was depicted, as the biopolymer was extracellular and phase separation occurred with the aid of organic solvents. The maximum cell dry weight was obtained 1.85 g/L.

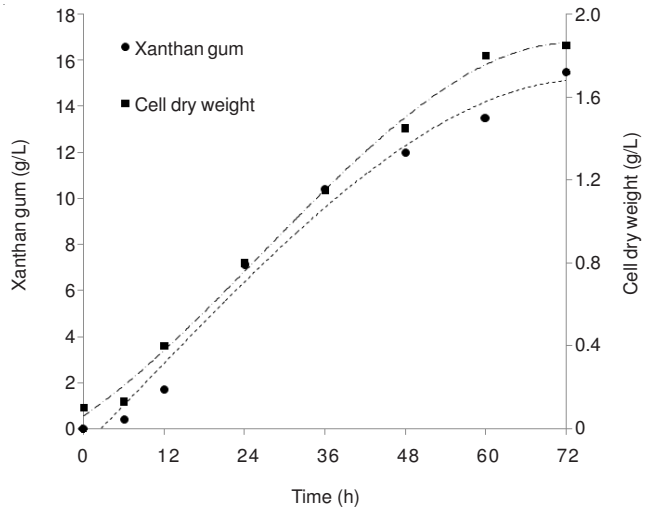


Fig. 1. (●) Xanthan gum production (g/L) & (■) cell dry weight (g/L) from palm syrup, operational conditions: temperature 30 ºC, agitation rate 500 rpm, initial pH = 7, initial concentration of palm syrup 30 g/L

Fig. 2 shows the effects of incubated culture temperature on xanthan gum production. The optimum temperature for maximum growth was obtained, since *X. campestris* showed the desired biological activities for xanthan production. Effect of temperature on xanthan production from glucose has been investigated by others 1,6.9, the optimum reported temperature was in the range 28-33 ºC. Maximum xanthan gum production from palm syrup in this research was obtained at 30 ºC.

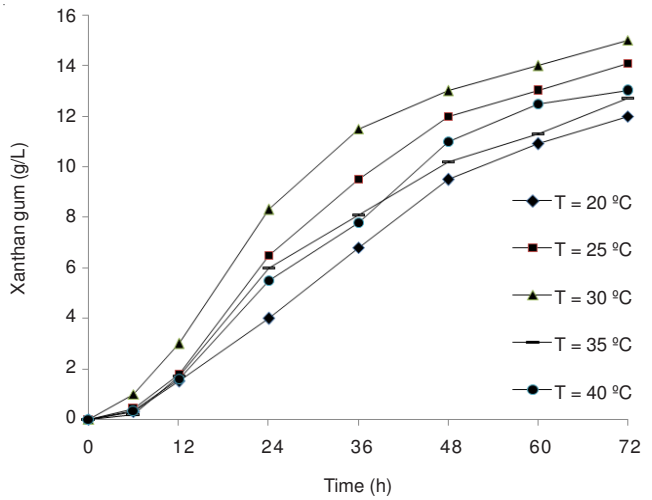


Fig. 2. Effect of fermentation temperature (20-40 ºC) on xanthan gum production

Fig. 3 demonstrates the apparent viscosities of the fermentation broth in different temperature. The cultured broth viscosities measured during the entire period of fermentation. The culture media apparent viscosity for palm syrup was gradually varied in increasing trend about 1 to 150 centipoises (cP), which in optimum temperature the maximum broth viscosity was achieved. The increasing trend in viscosity of

media was one of the indicating parameter to prove the production of xanthan gum.

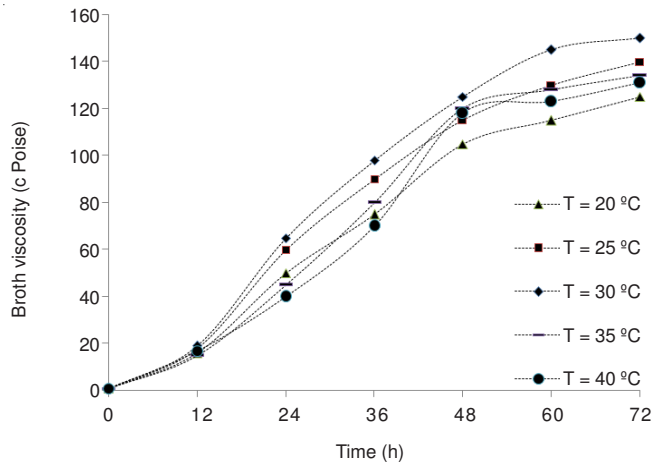
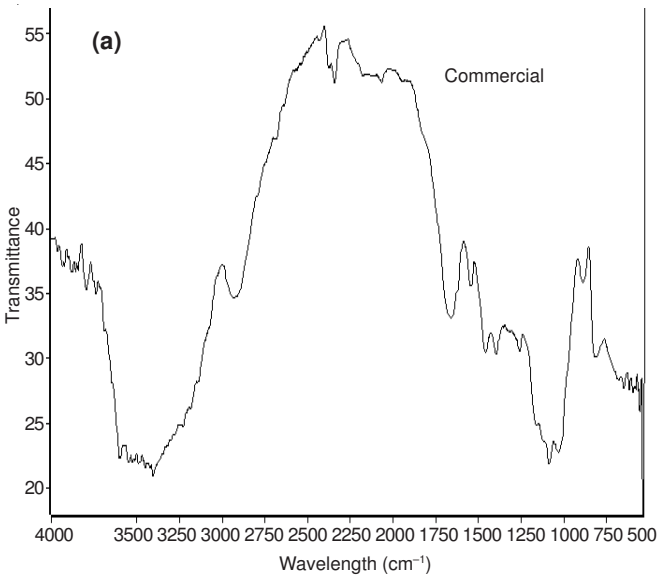


Fig. 3. Apparant viscosity of medium from palm syrup in different temperature (20-40 ºC)

FT-IR spectra of xanthan gum: The FT-IR spectra of synthesized xanthan gum *via* fermentation and commercial sample were considered under the same conditions. The FT-IR spectrum of the produced xanthan gum showed the quite similarity with the commercial sample functional groups such as carboxyl, carbonyl and acetal groups which were presented in Table-1. Fig. 4 summarizes and compares the wavelength spectra of commercial and synthesized xanthan. These results indicate the accommodating between synthesizes and commercial xanthan gum.

TABLE-1
FT-IR SPECTRAL DATA (cm^{-1}) OF STANDARD AND PRODUCT SAMPLES

Functional group	Carbonyl	Carboxyl	Acetal
Commercial xanthan gum	1627	1529	1160
Xanthan gum from palm syrup	1625	1527	1151



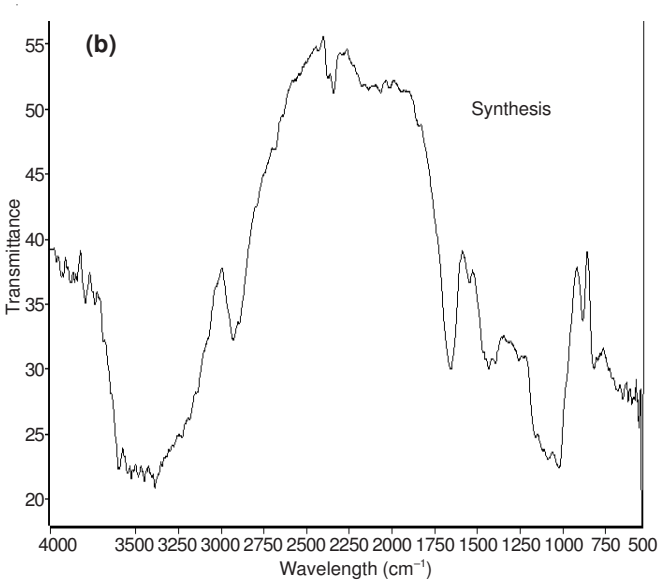


Fig. 4. FT-IR spectrum of commercial (a) and synthesized xanthan gum (b)

Conclusion

Xanthan gum was produced from palm syrup using *Xanthomonas campestris*. The effect of temperature fermentation was considered, while the other conditions were kept the same. The cultured broth viscosities and mass of cell dry weight measured during the entire period of fermentation. In addition, the bioproduct functional groups were compared with the commercial xanthan by FTIR spectra.

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