

# RICE STRAW HYDROLYSATE AS A PROMISING CULTURE MEDIUM FOR ASTAXANTHIN PRODUCTION BY THE RED YEAST *XANTHOPHYLLOMYCES DENDRORHOUS*

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## Abstract:

This research aimed to utilize rice straw as a low-cost substrate for astaxanthin production by *Xanthophyllomyces dendrorhous* TISTR 5730. Rice straw composed of high content of cellulose (35.92±0.51 %) and hemicellulose (21.13±1.93 %). After enzymatic hydrolysis with commercial cellulase, the rice straw hydrolysate contained the maximum of total reducing sugar content of 89.82±0.39 g/L. *X. dendrorhous* TISTR 5730 was cultivated on rice straw hydrolysate with 20 g/L of initial total reducing sugar as a culture medium. The maximum cell dry weight (5.47±1.45 g/L) with astaxanthin concentration (2.49±0.04 mg/L) was achieved. The kinetics parameters demonstrate the astaxanthin productivity of 0.50±0.01 mg/L/day with the astaxanthin coefficient of 0.13±0.00 mg/g sugar consumed. *X. dendrorhous* TISTR 5730 was capable to consume the reducing sugar in rice straw hydrolysate up to 97.08±0.03 %. The absorption spectra, HPLC profile and FTIR spectrum of the produced astaxanthin *X. dendrorhous* TISTR 5730 were characterized and compared to standard astaxanthin. This research indicated that rice straw hydrolysate could be used as a promising culture medium for astaxanthin production.

**Keywords:** Rice straw; Culture medium; Enzymatic hydrolysis; Astaxanthin production; *Xanthophyllomyces dendrorhous* yeast

## Introduction

Rice straw is produced from the harvesting of rice as agricultural residues. Generally, rice straw was used as animal feed, paper making and fertilizer (Zhu *et al.*, 2005). However, the limitation of used is reached due to the abundance of remained rice straw. The burning of rice straw is the only way to eliminate the unused rice straw. Resulting in a serious environmental problem by releasing a pollutant into the air and causing high risk for human health (Xiao *et al.*, 2001). The alternative way to use rice straw is challenged by converting into fermentable sugars. Rice straw is a lignocellulosic material which composed of high content of carbohydrate about 60 % by weight which consisted of cellulose (32 %-72 %), hemicellulose (19 %-27 %) and lignin (5 %-24 %) (Binod *et al.*, 2010). The cellulose in rice straw can be hydrolyzed by using enzyme into reducing sugar and further used as a fermentable sugar. The fermentable sugar is an interest carbon source for growth and produces the value-added compound of microorganisms. The utilization of rice straw as feedstock for microorganism is getting more attractive due to significantly decrease the production cost. Previously, rice straw was utilized to produce many high value-added substance such as ethanol, lipid, lactic acid, citric acid, and amino acid via fermentation processes (Zhang & Cai, 2008). Thus, rice straw might be a promising low-cost

substrate for producing high value bioactive compounds such as carotenoids, especially astaxanthin.

Astaxanthin is a lipophilic pigment that appearing as orange-red color. It is classified as xanthophyll carotenoid in which the structure contained oxygen. Astaxanthin structure composed of polyene chain joins with the  $\beta$ -ionone ring with a hydroxyl group and keto group in both ends (Ambati *et al.*, 2014). Due to its structure astaxanthin possess a powerful antioxidant activity by donating and scavenging free radical which 10 and 100 times greater than  $\beta$ -carotene and vitamin E, respectively (Higuera-Ciapara *et al.*, 2006). The properties of astaxanthin is responsible for immune response, oxidative stress prevention, anti-inflammatory and anti-aging (Yamashita, 2015). Astaxanthin is a naturally synthesized in a microorganism such as the yeast *Xanthophyllomyces dendrorhous* and microalgae *Haematococcus pluvialis*. The red yeast *X. dendrorhous* is one of the important sources for astaxanthin production owing to its ability to utilize low-cost substrate, high growth rate along with short production time and easy cultivation condition. In recent years, many researchers have been interested in cost-effective of astaxanthin production by *X. dendrorhous* from low-cost culture media. Several researchers presented the utilization of low-cost materials for astaxanthin production such as molasses (Haard, 1988), peat hydrolysate (Martin *et al.*, 1993), raw coconut milk (Domínguez-Bocanegra & Torres-Muñoz, 2004), mustard waste (Tinoi *et al.*, 2006), Mussel processing (Amado & Vázquez, 2015) wastewater and Sweet sorghum (Stoklosa *et al.*, 2019).

This research aimed to utilize rice straw hydrolysate as a culture medium for astaxanthin production via the fermentation process. It could be reduced the cost of production and applied as a culture medium for further scale-up of astaxanthin production.

## Materials and methods

Rice straw was collected from a field at San Sai, Chiang Mai, Thailand. Rice straw was dried and cut into small pieces. Then, the dried rice straw was ground and sieved through 60 mesh. The ground rice straw was stored in a plastic bottle.

### *Chemical Composition Determination*

The proximate analysis of rice straw for moisture, crude protein, crude lipid, fiber, total ash and carbohydrate contents was determined as described by the Association of Official Analytical Chemists (AOAC, 2005). Moisture content was measured by the drying method. Crude protein content was determined by the Kjeldahl method and crude lipid content was done by using Soxhlet extraction with hexane as solvent. Total ash content was incinerated in a furnace at 550°C. Fiber content was measured by weight difference methods. The carbohydrate content of rice straw was carried out by calculation. The lignocellulosic component of rice straw was also carried out by the detergent fiber analysis. Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and acid insoluble ash (AIA) were determined as described by Van Soest *et al.* (1991).

### *Rice straw hydrolysate preparation*

Rice straw was pretreated by alkali (30 % NaOH) pretreatment combined with an autoclave for 20 min as described by Kobkham *et al.* (2018). The pretreated rice straw was then hydrolyzed by commercial cellulase (iKnowZyme®, 119 FPU/mL) hydrolysis in citrate buffer solution (pH 4.8) at ratio 1:30 (w/v). The mixture was incubated in an incubator shaker at 50 °C with 150 rpm for 120 h. The reducing sugar content of rice straw hydrolysate was carried out by the

DNS method. The sugar composition was analyzed by High-performance liquid chromatography (HPLC).

### ***Astaxanthin Production on Rice Straw Hydrolysate***

*Xanthophyllomyces dendrorhous* TISTR 5730 was obtained from the Thailand Institute of Scientific and Technology Research (TISTR). The seed culture was prepared in the YM medium for 36 h. Then, 10 % of *X. dendrorhous* TISTR 5730 seed culture was inoculated into rice straw hydrolysate containing 20 g/L of total reducing sugar concentration. The mixture was incubated in an incubator shaker at 20 °C with 200 rpm for 10 days. The culture was taken every day for cell dry weight, astaxanthin content and total reducing sugar concentration determinations.

### ***Analytical methods***

The cell dry weight was determined using the gravimetric method and calculated in terms of concentration (g/L). The astaxanthin content was estimated via cell disruption and solvent extraction according to Urnau *et al.* (2018). The astaxanthin content was represented in terms of astaxanthin concentration (g/L) and yield (g/g dry cell basis).

The characteristics of astaxanthin were analyzed and compared to standard astaxanthin. The adsorption spectra of astaxanthin were scanned by a UV/VIS spectrophotometer (Thermo Scientific Evolution™ 201) with a wavelength in the range of 300 – 800 nm. The HPLC analysis of astaxanthin extract was performed by using the Hewlett Packard 1100 Series HPLC system coupled with a UV detector at 474 nm. The functional structure of astaxanthin was analyzed by the Fourier transform infrared spectroscopy (FTIR) (Bruker, USA). The sample was measured in ATR mode with the scanning wavenumber in the range of 4000 to 500 cm<sup>-1</sup>.

## **Results and discussion**

### ***Chemical Composition of Rice Straw***

The chemical composition of rice straw was determined and shown in Table 1. The moisture content of rice straw was found to be 7.70±1.88 % which is slightly higher than the moisture content of rice straw about 5.40±0.20 % from the study of Ma *et al.* (2009). The low moisture content of rice straw would enhance the storage stability and extend the shelf life for further utilization. The crude protein was 4.17±0.26 % and crude lipid was found in a trace amount of 0.93±0.29 %. These results were correlating to the report of Malik *et al.* (2015), who exhibited the protein and crude lipid contents of rice straw as 4.5 and 1.0 %, respectively. Ash content contained about 15.72±0.26 % which the results were similar to 14.56±0.43 and 16 % of ash content (Harun & Geok, 2016; Malik *et al.*, 2015). Moreover, 32.11±1.56 % of the carbohydrate content in rice straw was found and the results corresponded to the previous research of Candia-García *et al.* (2018), who obtained the carbohydrate content of rice straw in Columbia about 31.34±0.24 %. The fiber content was 39.37±2.23 % and the results in this study were higher than with the fiber content of 29.8 % in rice straw from the previous reported by Malik *et al.* (2015). The fiber in rice straw was considered an important component because it contained the lignocellulosic composition. The results presented that rice straw contained cellulose, hemicellulose and lignin of 35.92±0.51, 21.12±1.93 and 5.42±0.35, respectively. Kobkham *et al.* (2018) revealed that the lignocellulosic content of rice straw was 34.40±0.03, 26.68±0.08 and 7.30±0.08 % of cellulose, hemicellulose and lignin, respectively. While Binod *et al.* (2010) also found a similar content of cellulose and hemicellulose about 38 and 19.7 %, respectively.

respectively. As the results of this research, rice straw could be utilized as the culture medium for microbial growth and fermentation. The mainly composed of cellulose and hemicellulose was suitable material for further hydrolysis to obtain the fermentable sugar.

**Table 1:** The chemical composition of rice straw

<b>Chemical composition</b>	<b>Content (% , w/w)</b>
Moisture	7.70±1.88
Crude lipid	0.93±0.29
Crude protein	4.17±0.26
Carbohydrate	32.11±1.56
Ash	15.72±0.26
Fiber	39.37±2.23
Cellulose	35.92±0.51
Hemicellulose	21.13±1.93
Lignin	5.42±0.35

Data expressed as mean±SD of triplicate experiments.

### ***Enzymatic hydrolysis of rice straw***

The rice straw was pretreated by using alkaline pretreatment with sodium hydroxide solution for delignification and increased surface area of cellulose. The main effect of sodium hydroxide on delignification demonstrates the breaking the ester bonds cross-linking lignin and hemicellulose, resulting in increasing the porosity of rice straw structure (Binod *et al.*, 2010). The pretreated rice straw composed mainly of cellulose which could be hydrolyzed into glucose. This research performed the enzymatic hydrolysis of rice straw by using commercial cellulase. The rice straw cellulose was hydrolyzed by 119 FPU/ml of cellulase at 50°C for 120 h of hydrolysis time. The reducing sugar concentration of 89.82±0.39 g/L and yields of 0.71±0.01 g/g rice straw was found to be in rice straw hydrolysate. Comparison to the previous studies, the results of this research were higher than the reducing sugar content from Ong *et al.* (2012). The results showed that the fermentable sugar content of 3.62 g/L was obtained from 10 U/g of cellulase for 96 h. Takano & Hoshino (2018) reported that the pretreated rice straw by using alkali pretreatment was hydrolyzed by enzymatic hydrolysis and revealed that fermentable sugar content of rice straw hydrolysate was achieved of 75.3 g/L. Moreover, the rice straw hydrolysate composition was investigated by using HPLC analysis and the results were shown in Table 2. The results represented that rice straw hydrolysate contained a high amount of glucose (82.35±2.23 %) which was broken down from the cellulose structure. However, the xylose (3.46±0.11 %) and arabinose (0.72±0.03 %) were also detected in a small amount. These pentose sugars was hydrolyzed from the hemicellulose which remained in during the pretreatment process. However, the unidentified saccharide of rice straw hydrolysate was also found about 13.47±1.67 % of total reducing sugar. These results were complementary with Xiao *et al.* (2001) who reported that the rice straw hydrolysate composed of glucose (81.55 %), xylose (15.67 %) and arabinose (2.73 %).

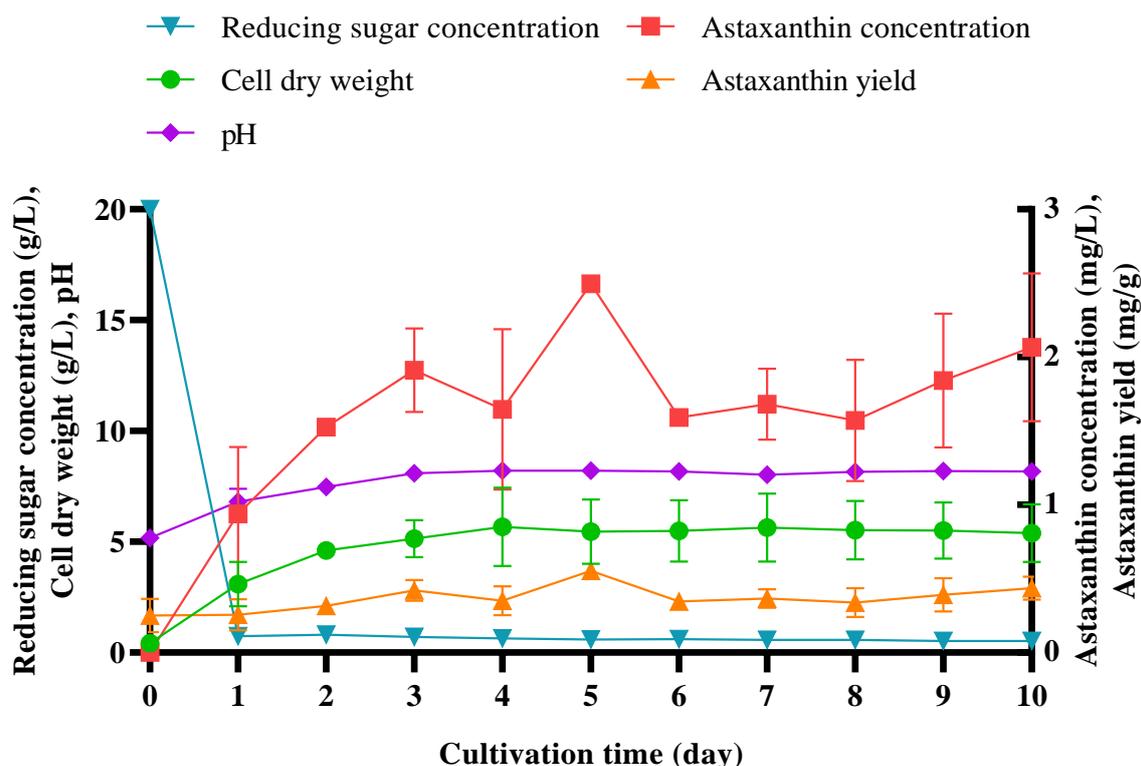
**Table 2:** Total reducing sugar content and sugar composition of rice straw hydrolysate after enzymatic hydrolysis

<b>Total reducing sugar content</b>	
Total reducing sugar concentration (g/L)	89.82±0.39
Total reducing sugar yield (g/g rice straw)	0.71±0.01
<b>Sugar composition (% of total reducing sugar)</b>	
Glucose	82.35±2.23
Xylose	3.46±0.11
Arabinose	0.72±0.03
Others	13.47±1.67

Data expressed as mean ± S.D of triplicate experiments.

### ***The growth and Astaxanthin production on rice straw hydrolysate***

The red yeast *X. dendrorhous* TISTR 5730 was cultivated on rice straw hydrolysate containing total reducing sugar of 20 g/L with the initial pH of 5.2 for 10 days of cultivation. The initial of total reducing sugar at 20 g/L was selected as the results of the prior study by Tinoi *et al.* (2006). Besides, Lui and Wu (2007) confirmed that glucose concentration of approximately 20 g/L was suitable for astaxanthin production by *X. dendrorhous* TISTR 5730. Figure 1 indicates the growth of *X. dendrorhous* TISTR 5730, astaxanthin production and remained reducing sugar concentration. At the early of cultivation, the results revealed that the reducing sugar was consumed up to 96.3±0.28 % within the first day and kept constant with the rest of the cultivation time. After the reducing sugar was consumed as a carbon source for yeast cell metabolism. The yeast cell was grown and increased to a maximum of 5.47±1.45 g/L in 4 days. Then, the cell dry weight of *X. dendrorhous* TISTR 5730 remained in the stationary state until 10 days of cultivation. Due to nutrients for growth containing in rice straw hydrolysate was exhausted and became into the stress condition. Therefore, the astaxanthin was initiated to accumulate inside the *X. dendrorhous* TISTR 5730 cell and the highest astaxanthin yields were 0.56±0.02 mg/g with astaxanthin concentration of 2.49±0.04 mg/L at 5 days of cultivation. Astaxanthin is considered as the secondary metabolites produced during the stationary phase of the *X. dendrorhous* cultivation. The production of astaxanthin by *X. dendrorhous* recognized as growth associated metabolite (Liu & Wu, 2007). In this research, astaxanthin was synthesized when the reducing sugar of rice straw hydrolysate was consumed and the lack of nutrients occurred after the highest of cell dry weight concentration. Liu & Wu (2007) reported that astaxanthin content also reached the maximum due to the stress condition around the *X. dendrorhous* cells. The initial pH of the rice straw hydrolysate medium was 5.2 which is slightly acidic condition. After the first day of cultivation, pH was increased to neutral and remained constant in neutral condition around 7.0 - 8.4. The previous studied confirmed that the suitable initial pH for *X. dendrorhous* TISTR 5730 cultivation was around 5.0 - 7.0 and considered as neutral pH (Martin *et al.*, 1993).



**Figure 1:** Time course of the growth and astaxanthin production of *X. dendrorhous* TISTR 5730 on rice straw hydrolysate at 20 g/L with 10 % of initial inoculum for 10 days of cultivation

Table 3 presented the kinetic parameters of astaxanthin production on rice straw hydrolysate by *X. dendrorhous* TISTR 5730. The maximum specific growth rate was  $0.39 \pm 0.00$  day<sup>-1</sup> and biomass yield was  $0.23 \pm 0.01$  g/g of total reducing sugar consumption. While the cellular astaxanthin reached to  $551.92 \pm 22.11$  µg/g with the productivity of  $0.50 \pm 0.01$  mg/L/day. The astaxanthin coefficient was  $0.13 \pm 0.00$  mg/g sugar consumed. Furthermore, the sugar consumption rate was  $3.88 \pm 0.01$  g/L/day and sugar consumption was up to  $97.08 \pm 0.03$  % within 5 days of cultivation. This research indicated that rice straw hydrolysate could be used as a promising culture medium which mainly composed of glucose with the small amount of xylose and arabinose for astaxanthin production by *X. dendrorhous* TISTR 5730. According to Xu *et al.* (2004) demonstrated that *X. dendrorhous* could be grown on the different type of sugar such as glucose, xylose, arabinose, cellobiose, maltose and sucrose. The medium containing glucose, galactose and cellobiose was revealed to obtain a high biomass concentration corresponding with the high astaxanthin content than others media which led to high astaxanthin productivity. However, a high glucose concentration could undergo inhibition of astaxanthin synthesis within the yeast cell which leads to overflow to increase ethanol production (Xiao *et al.*, 2015). Parajó *et al.* (1998) represented that the eucalyptus hydrolysate supplement with peptone was used for *X. dendrorhous* cultivation and astaxanthin production. The results showed that astaxanthin concentration of 2.14 mg/L with astaxanthin content of 448 µg/g was obtained. While Hayman *et al.* (1995) displayed the astaxanthin concentration produced by *X. dendrorhous* on corn wet-milling was up to 2.6 mg/L with the astaxanthin content of 400 µg/g. Moreover, Yang *et al.* (2011) presented the astaxanthin concentration was 2.98 mg/L when *X. dendrorhous* was cultured on cassava hydrolysate.

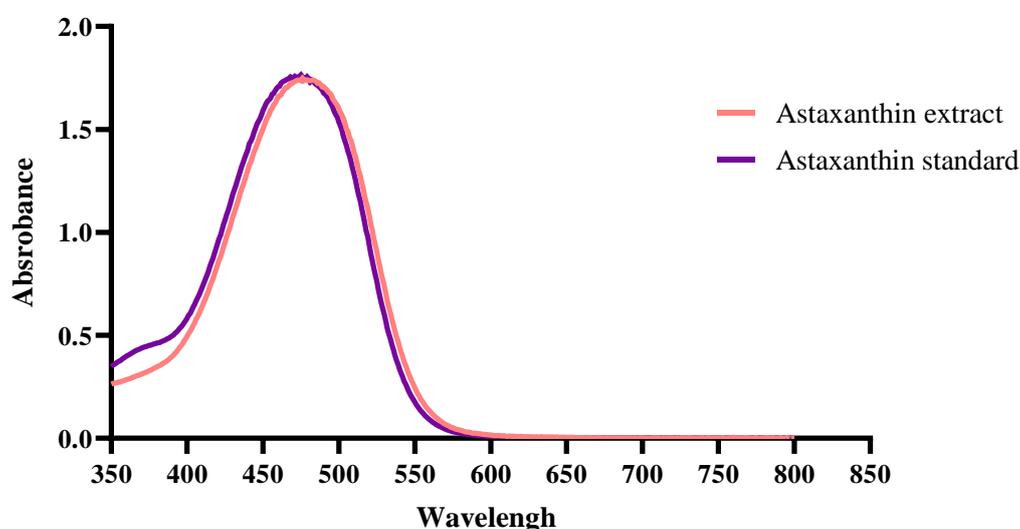
**Table 3:** Kinetic parameters of astaxanthin production on rice straw hydrolysate by *X. dendrorhous* TISTR 5730 for 5 days of cultivation

Parameters	
<i>X. dendrorhous</i> TISTR 5730 growth:	
X (g/L)	5.47±1.45
$\mu_{\max}$ (day <sup>-1</sup> )	0.39±0.00
R <sub>X/S</sub> (g/g)	0.23±0.01
Astaxanthin production:	
concentration (mg/L)	2.49±0.04
cellular (µg/g)	551.92±22.11
productivity (mg/L/day)	0.50±0.01
coefficient (mg/g sugar consumed)	0.13±0.00
Sugar concentration:	
Initial concentration (g/L)	20.00±0.00
Final concentration (g/L)	0.59±0.05
Sugar consumption rate (g/L/day)	3.88±0.01
% Sugar consumption	97.08±0.03

Data expressed as mean ± S.D of triplicate experiments.

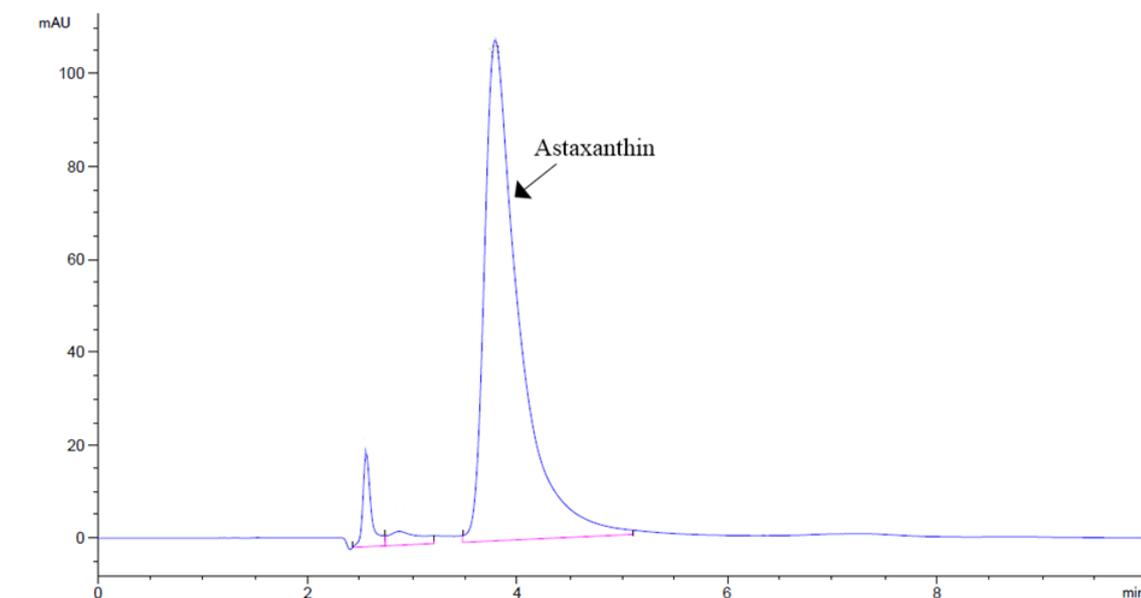
### *The characterization of astaxanthin*

The absorption spectra of produced astaxanthin by *X. dendrorhous* TISTR 5730 were analyzed in the wavelength between 350-800 nm and compared to the astaxanthin standard. Figure 2 shows the astaxanthin absorption spectra. The absorption spectra profile of produced astaxanthin presented to similar the astaxanthin standard. The maximum absorbance demonstrated at the wavelength of 474.90 nm for standard astaxanthin and 476.02 nm for the astaxanthin from *X. dendrorhous* TISTR 5730. The results indicated that the produced astaxanthin was identical to the standard astaxanthin.



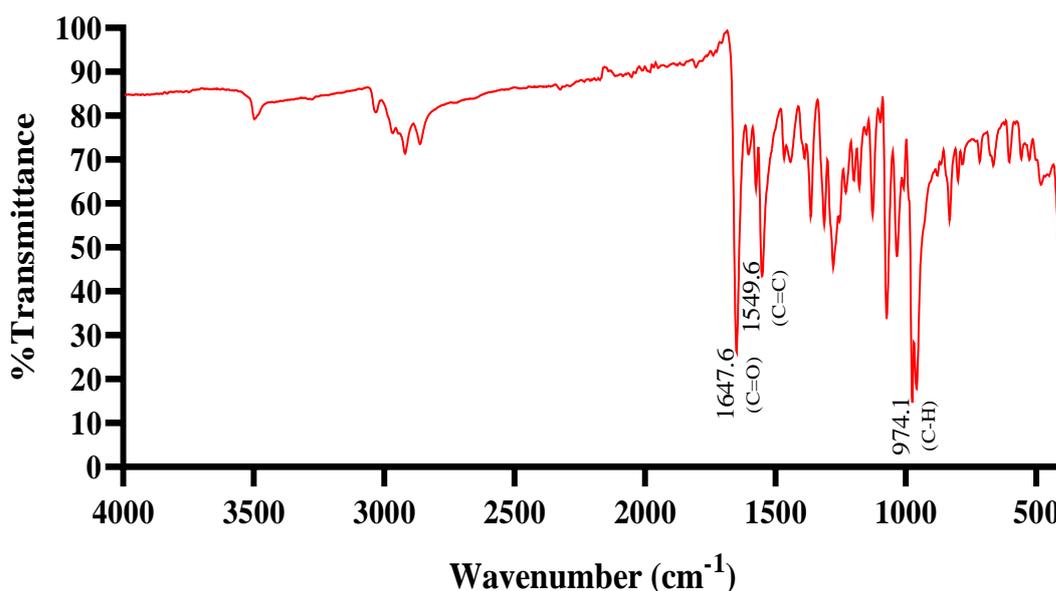
**Figure 2:** The absorption spectra of the standard astaxanthin and produced astaxanthin by *X. dendrorhous* TISTR 5730 when cultured on rice straw hydrolysate

The HPLC analysis of the produced astaxanthin by *X. dendrorhous* TISTR 5730 was performed and revealed that the retention time was at 3.788 min as shown in Figure 3. The HPLC chromatogram presented only one peak of the astaxanthin compound as a major component. According to Schmidt *et al.* (2011) revealed that *X. dendrorhous* produced astaxanthin as a dominant compound up to 87 % of the carotenoid product.



**Figure 3:** HPLC chromatogram of astaxanthin profile from *X. dendrorhous* TISTR 5730 production from rice straw hydrolysate

The FTIR spectrum of astaxanthin was recorded as the transmittance (%) versus wavenumber in the range of  $4000 - 400 \text{ cm}^{-1}$  to determine the functional group contributions as shown in Figure 4. The spectrum comprised the strong absorption band at  $1647.6 \text{ cm}^{-1}$  corresponding to C=O stretching vibration which presented in the terminal ring of  $\beta$ -ionone. The absorption band at  $1549.6 \text{ cm}^{-1}$  corresponded to the C=C stretching vibration of the aromatic ring (Liu *et al.*, 2019). And at  $974.1 \text{ cm}^{-1}$  attributed to the C-H in the C and C conjugate system. All the absorption band of astaxanthin in these results was agreed with the previous study of (Kaga *et al.*, 2018).



**Figure 4:** FTIR spectrum of the produced astaxanthin by *X. dendrorhous* TISTR 5730 when cultured on rice straw hydrolysate

## Conclusion

This research represented that rice straw composed of high content of cellulose and hemicellulose. The hydrolysate of rice straw was obtained by enzymatic hydrolysis and gave a high concentration of reducing sugar-containing with glucose as dominant sugar and also pentose sugars (xylose and arabinose). The 20 g/L of initial reducing sugar concentration of rice straw hydrolysate was applied as a culture medium for *X. dendrorhous* TISTR 5730. The successful growth and high astaxanthin production by *X. dendrorhous* TISTR 5730 was obtained. Thus, rice straw hydrolysate could be used as a promising culture medium in astaxanthin production *X. dendrorhous* TISTR 573 for reducing the cost of production and can be used in the further large-scale production.

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