



## Xylitol Production from Agricultural Wastes by *Candida tropicalis*

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**Abstract:** Xylan produced various agricultural residues including wheat (Furat, Abugraib and Abaa), Papyrus and Sunflower stalks in different ways, including the use of diluted acid, dilute base and self-degradation. The results showed that the acidic method in the production of xylan from various agricultural residues compared with other methods was superior, the highest quantity of xylan  $187.6 \mu\text{g}\cdot\text{ml}^{-1}$  was obtained from the agricultural waste of Papyrus, while it was  $157.6$ ,  $157.6$ ,  $161.6$  and  $161.3 \mu\text{g}\cdot\text{ml}^{-1}$  of wheat category of furat, wheat Abu Ghraib, wheat Abaa and sunflower stalks respectively, based on the results obtained, the xylan produced by the acidic method of the different agricultural residues was selected to determine the optimal carbon source for production of xylanase using bacteria *Bacillus subtilis* strain RS1 locally isolated. After the production of xylitol, the descriptive diagnosis was performed using an HPLC device, depending on the time of the 38.4 minute time lapse reaction of the standard Xylitol and compared with the time of the production of *Candida tropicalis*, the amount of the processed xylitol was  $8.3 \mu\text{g}\cdot\text{ml}^{-1}$ , the calculated xylitol was compared standard xylitol.

**Keyword:** Agricultural waste, Xylanase enzyme, Submerged fermentation.

## Introduction

Xylitol ( $\text{C}_5\text{H}_{12}\text{O}_5$ ) is a five carbon sugar polyalcohol, obtained from xylose, it is found in small amount in various fruits, vegetables, algae and mushrooms, it is sweetening power similar to that of sucrose, having one third less calorie content (Walsh *et al.*, 2018). Xylitol is used as an alternative sweetener in the food, pharmaceutical and personal care, it has lower energy (about 40% fewer calories than the amount found in sucrose), it absorbs slowly from digestive tract and enters the

metabolic pathway independently of insulin. So used as a sweetener in dietetic food for diabetics (Mohamad *et al.*, 2015). Xylitol is utilized in the dental industry, as anti-cariogenic and biomedical properties (Vallejos *et al.*, 2016).

Industrially xylitol is produced by catalytic hydrogenation of D-xylose under high temperature and pressure, biotechnological xylitol production is a replacement for chemical process, as it occurs under milder

process conditions and utilizes agricultural and forestry wastes which offer the possibilities of economic production of xylitol (Dasgupta *et al.*, 2017). Xylose is produced from agricultural material (hemicellulose fraction) by enzymatic and chemical hydrolysis and converted to xylitol by yeast strain belonging to *Candida* genus (Asada *et al.*, 2015). The aim of this study is produce a natural sweetener (xylitol) with low calories from agricultural wastes.

## **Materials & Methods**

### **Extraction of xylan from Papyrus, Wheat Furat, Wheat Abugraib, Wheat Abaa and Sunflower stalks**

Five different strategies were applied to each biomass (papyrus, wheat furat stalks, wheat abugraib stalks, wheat abaa stalks and sunflower stalks) in order to recover maximum amount of xylan from raw biomass.

#### **Extract of xylan with diluted acid**

According to the diluted acid extract was applied with slight modification of method of Yang *et al.* (2005). biomass was diluted acid with (0.01 M H<sub>2</sub>SO<sub>4</sub>) and incubated at 60 °C for 12 h. The with distilled water until draw and dried in the oven, then distilled water was added to the biomass by 1:3 (w/v) and put the mixture in autoclave for 1 h, dry the output and grinding in an electric mill to obtain xylan powder.

#### **Extract of xylan with diluted alkali**

The mixture was drain by mixing 50 g of biomass separately with 80 ml of 1.25 M NaOH for 10-15 min. then the mixture incubated in a shaker with 150 rpm at 37 °C for 3 h and centrifuged at 8500 x g for 20 min, taking the filtrate modulation to pH 5 with concentrated HCl and using as substance

mainly for enzymatic degradation (Yoon *et al.*, 2006).

### **Extraction of xylan by autohydrolysis**

The biomass was soak separately with distilled water in a ratio of 1:8 (w/v) and the slurry was autoclaved at 121 °C and 15 lbs for 1 h, mix the mixture with muslin cloth, adjust the filtrate and pH 5 using concentrated HCl and use substance mainly for enzymatic degradation (Tan *et al.*, 2008).

### **Quantification of xylan extracted from agricultural wastes**

Xylan was quantified extracted according to the method reported by (Dubois *et al.*, 1956), based on the of method phenol-sulfuric acid to estimate total sugars represents the amount of xylan extracted from the biomass.

### **Microorganism**

*Bacillus subtilis* bacteria isolated locally from soil, which produce xylanase enzyme, were used to convert xylan to xylose, the pH was adjusted to 7 and the infestation was sterilized and the production was irrigated by 1 ml of bacterial suspension by  $1 \times 10^7$  cell.ml<sup>-1</sup> an incubated in an 37 °C and shaking incubator and 150 rpm for 48 h, and then used a *Candida tropicalis* yeast and obtained from Iranian Biological Resource Center (IBRC) in Tehran, producing xylose reductase enzyme to convert xylose to xylitol, the pH was adjusted to 6 and the infestation was sterilized and the production was irrigated by 10 ml of yeast suspension by  $1 \times 10^6$  cell.ml<sup>-1</sup> an incubated in a 30 °C and shaking incubator and 150 rpm for 48 h.

### **Identification of Xylitol by High Performance Liquid Chromatography (HPLC)**

High performance liquid chromatography was used in the identification of xylitol production

by *Candida tropicalis* yeast (Rao *et al.*, 2008). This method was used in the qualitative and quantitative estimation of xylitol as follows:

Qualitative and quantitative estimation of xylitol using the 8×300 mm Eurokat H column and the refractive index (RI) for alcoholic sugars, the size of the injected model was 20 µl, use the high performance liquid chromatography for the chemistry laboratory, Department of Food Science, Faculty of Agriculture, University of Ferdowsi.

The test was performed using the mobile phase of the solution of 100% sulfuric acid

(pH 2.0 ± 0.1). the separation was performed at room temperature at flow rate of 0.15 ml.min<sup>-1</sup>.

## Result & Discussion

### Extraction of xylan from Agricultural wastes

Table (1) showed that the acid yield in the extraction of xylan from agricultural waste compared to other methods, and the highest quantity of xylan 187.6 µg.ml<sup>-1</sup> obtained from the agricultural waste of papyrus, while 157.6, 157.2, 161.6 and 161.3 µg.ml<sup>-1</sup> of wheat furat, wheat abugraib, wheat abaa and sunflower stalks respectively.

**Table (1): Quantity of xylan extracted from different agricultural waste using different methods.**

Agricultural wastes		Quantity of Xylan (µg.ml <sup>-1</sup> )		
		Dilute Acid	Dilute Alkali	Autohydrolysis
Wheat	Furat	157.6	89.3	78.1
	Abugraib	157.2	88.1	75.2
	Abaa	161.6	93.7	82.2
Papyrus		187.6	102.3	94.6
Sun Flower Stalks		161.3	90.5	82.3

There has been a lot of research on the extraction of xylan from agricultural waste for use in the production of xylanase enzyme, Sporck *et al.* (2017) used alkali and enzymatic methods in extracting xylan from sugar cane and found superiority of the alkali method with a recovery rate of xylan 53%, in the enzymatic method 22%, while Hauli *et al.* (2013) used the alkali method in extracting the xylan from different sources and obtained different retrieval rates, the highest was sugar cane with 49% recovery, 42% wheat stalks,

and Ratanadewi *et al.* (2016) from the extraction of xylan from the waste of beans using the alkali method.

### Identification of xylitol by High Performance Liquid Chromatography

The descriptive diagnosis was performed using on HPLC device, depending on the time on the 38.4 min time lapase reaction of the standard xylitol in fig. (1) and comparing it with the time of the production of *Candida tropicalis* as shown in fig.(2) by 8.3 µg.ml<sup>-1</sup>.

Several researchers used HPLC in the diagnosis of xylitol. Rao *et al.* (2008) reported using in the diagnosis of xylitol produced using *Candida tropicalis* OMV5 using SHDEX SC 1011 8×300 mm column and detection WATERS 410 using mobile phase using water at 80 °C flow rate of 0.5 ml.min<sup>-1</sup>,

while Furlan & de Castro (2001) identified the diagnosis of xylitol using HPLC using *Candida tropicalis* using the Interaction Ion 300 column, the mobile phase of the hydrochloric acid solution was 0.025 molar, while Hernandez-Perez *et al.* (2016)

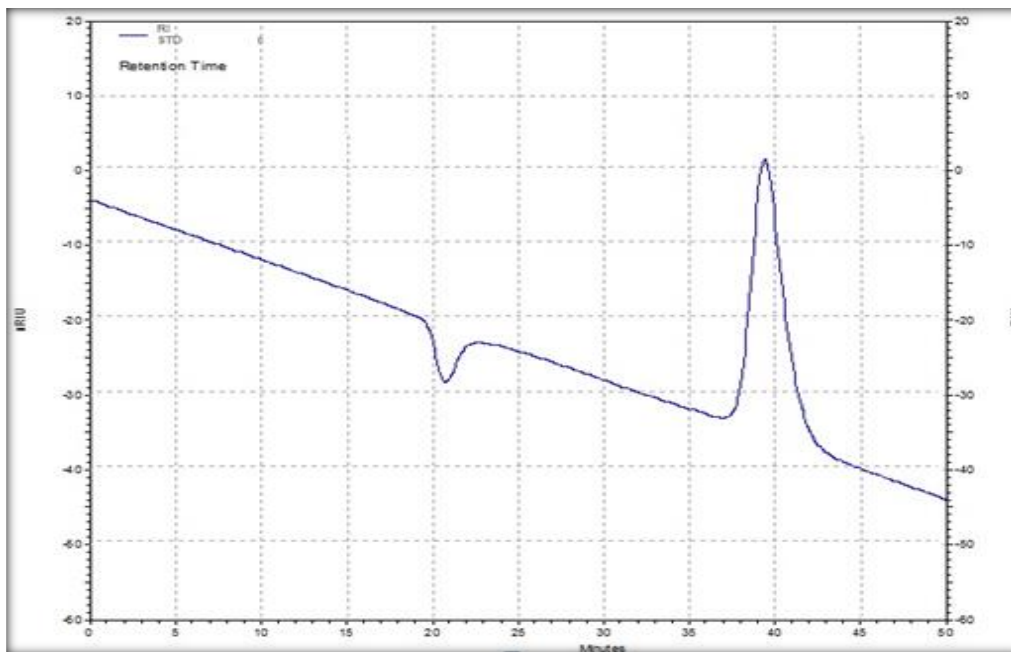
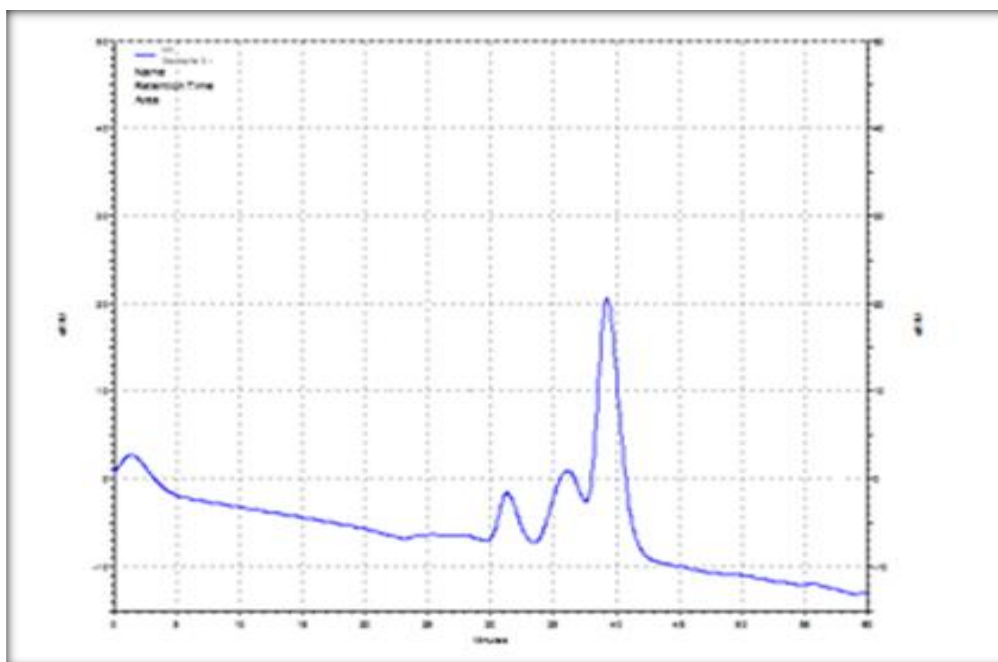


Fig. (1): High Performance Liquid Chromatography of the standard xylitol sample.



**Fig. (2): High Performance Liquid Chromatography produced from *Candida tropicalis* yeast using xylitol produced from papyrus xylan, the calculated xylitol was compared to standard xylitol.**

identified the diagnosis of xylitol, xylose, glucose, arabinose, acetic acid, glycerol and ethanol using HPLC using *Candida guilliermanii* yeast using the Aminex HPX-87 column and the Refractive Index and conducting the test using the mobile phase of the water solution for sulfuric acid 0.01 N at 45 C and a flow rate 0.5  $\mu\text{g}\cdot\text{min}^{-1}$ .

### Conclusions

The best method for extracting xylan is the acidic method compared to the alkali method and the method of autohydrolysis using different sources of agricultural residues, and the diagnosis of xylitol by High Performance Liquid Chromatography (HPLC).

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