ENZYME-ASSISTED EXTRACTION OF ANTHOCYANINS FROM PURPLE TOTOMOXTLE (CORN HUSK)

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Introduction. Totomoxtle (corn husk) is agricultural and industrial waste (fig 1). Its composition is 43.2% arabinoxylan and 21.8% cellulose[1]. Xylan is a linear polymer of β-D-xylopyranosyl units linked by (1-4) glycosidic bonds. Their hydrolysis is done using xylanases. These enzymes are produced by bacteria, yeast, seed, etc., but the principal source is fungi[2]. So xylan is the principal type of polymer in cell wall. In specific case of purple corn, which is from Mexico/Mesoamerica, their husk could be an important source of anthocyanins but first is needed to hydrolyzer the cell wall, for the purpose of optimized the extraction.

Fig 1. Maize plant with totomoxtle

Anthocyanins present a spectrum from orange to blue in colour in the natural world. They possess known pharmacological properties. In this way, anthocyanins have been noted not only as a food colorant but also as a health food material. Their instability is a disadvantage in food industry and stability is improved by acylated anthocyanins[3].

The anthocyanins have been characterized in purple corn, including cyd-3-dimalgluc, cyd-3-gluc, pg-3-gluc and pn-3-gluc[4]. The aim of this study is evaluate *Aspergillus flavus's* enzymes to enhance the anthocyanin extraction and enhance the acylated anthocyanin extraction.

Methods. Totomoxtle was obtained from Ixtenco-Tlaxcala. All the material was ground to a particular size of 0.24 mm. Totomoxtle was extracted with acid ethanol and the waste was subjected to enzymatic treatment. *Aspergillus flavus* was grown in cobcorn, after that, the enzyme was extracted; the principal activity is xylanases, pectinases and cellulases (1170 U/g). The most important parameters were evaluated and these were concentration, pH, temperature and time. Xylanases were compared with a commercial enzyme, Celluclast (700 U/g). It was bought from Sigma-Aldrich. Monomeric anthocyanin content was determined using pH differential method[5]. Finally, anthocyanins profile was determined by HPLC analysis[6]. All the experiments were carried out in triplicate and average values were reported. The enzymatic extraction was compared with ultrasonic, microwave and maceration methods.

Results. As shown in table 1, the extraction yields of total anthocyanins did not increase with higher enzyme concentration but pH 5 improve the yield in 42 %.

On the other hand, as shown in table 2, when the temperature was higher the anthocyanins were degrading. For that reason, although optimum temperature was 60°C the best temperature to anthocyanins extraction is 30°C.

Table 1 Concentration of anthocyanins of extracts using different enzyme concentration and pH (mg/100 g $_{FW\ (Fresh\ Weight)}$)

pH	Without enzyme	1% enzyme (p/v)	1.5% enzyme (p/v)	2% enzyme (p/v)
3.citrate's buffer	42±1.0	48±1.0	48±1.0	42±1.0
5.phosphate's buffer	54±3.0	77±1.0	71±3.0	71±3.0

Table 2 Concentration of anthocyanins of extracts in different temperatures (mg/100 g $_{FW}$)

Without enzyme **30°**	**30°C**	Without enzyme 40°	**40°C**	Without enzyme 50°	**50°C**
54±1.0	77±1.0	38±0.5	52±3.0	29±0.5	35±0.5

According to table 3, there was not difference between xylanases and commercial celluclast except in extraction time. These results show that xylanases achieved yield faster than commercial enzymes.

Table 3 Concentration of anthocyanins of extracts in different time using two enzymes, xylanases vs. commercial enzymes (mg/100 g $_{FW}$)

Time (h)	Xylanases	Celluclast
0	4±0.2	5±0.06
6	82±0.3	72±0.2
12	81±0.7	81±0.5
24	74±0.8	76±0.6

First extraction of anthocyanins from purple corn was 2189 mg/100g$_{FW}$ and the waste was 87 mg/100g$_{FW}$, this increase of 4%, however, the ultrasonic and microwave method is better than enzyme-assisted extraction in 13%.

HPLC profile showed 6 anthocyanins, the major was cyanidin-3-(6-malonyl)glycoside and cyaniding-3-glucoside with all methods, but enzyme-assisted extraction increased the acylated anthocyanin, ergo the anthocyanins in wall cell are acylated so when the cell wall is hydrolyzed there are more acylated anthocyanins.

Conclusions. The best conditions to extract anthocyanins with xylanases were 1% of enzyme in pH 5, temperature 30°C and 6 hours. Anthocyanin extraction from waste of totomoxtle was better with xylanases from *Asperigillus flavus* than celluclast (commercial enzyme). The enzymes-assisted extraction enhanced the yield in 4%, and also was higher the acylated anthocyanins.

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References.

1. Masahiro K, Wataru K, Kazuya O. and Toshiaki K. (2001). *Applied Biochemistry and Biotech.* 90: 251-259.
2. Polizeli M., Rizzatti A., Monti R., Terenzy H. and Amorim D. (2005). *App Microbiol Biotech*. 67: 557-591.
3. Bakowska-Barezak A. (2005). *Polish J. of Food and Nutrition Science* 14/55(2):107-116.
4. Zhendong Y. and Weiwei Z. (2010).*Innovative Food Science and Emerging Tech.* 11: 169-176.
5. Giusti M. and Wrolstad R. (2001). Current Protocols in Food Analytical Chemisty F1.2.2-F1.2.13
6. Rune S., Atle A. and Øyvind M. (1999).*Phytchemistry.* 50: 1081-1086