



# AMARANTH STARCH AS CARBON SOURCE TO PRODUCE CGTase BY MEANS OF SUBMERGED FERMENTATION UTILIZING *Bacillus megaterium* AND ITS KINETIC CHARACTERIZATION

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**Introduction.** Starch is a polymer with a long potential as raw material to obtain added value products. During starch degradation by the action of bacterial enzyme, cyclodextrin glycosyltransferase (CGTasa) (1) is obtained. This enzyme can form compounds called cyclodextrins (CD) which are widely used in food industry to form inclusion complexes. The aim of this research was to observe that amaranth starch is a good carbon source for *B. megaterium* to produce CGTase as well as a good substrate to yield  $\beta$ -cyclodextrins.(3).

**Methods.** Amaranth starch was obtained by two different methods: First it was obtained using 1%: sodium hydroxide (NaOH) and 1% sodium bisulfite (NaHSO<sub>3</sub>). *Bacillus megaterium* strain was spread in culture media with two different carbon sources: amaranth starch (AS) and corn starch (CS). Two thermic pre-treatments were assayed for amaranth starch (60 and 90° C) before the culture media was prepared. CGTase was obtained by liquid fermentation; kinetic parameters ( $\mu$ ,  $t_d$ ,  $Y_{X/S}$ ,  $Y_{E/X}$ ,  $Y_{E/S}$ ,  $P=X_{max}/t$ ,  $P=E_{max}/t$ ) were evaluated. The enzymatic activity was assessed quantifying the  $\beta$ -CD production as was described by Mäkelä (1987).

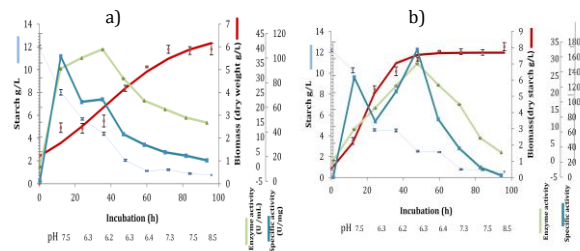
**Results.** The yield of starch coming from amaranth was significantly greater than that of corn starch (52.47%  $\pm$  3.24 and 40.25  $\pm$  1.57 respectively) when sodium hydroxide was used ( $p \leq 0.05$ ). Obtained starch characterization is shown on table 1.

**Table 1.** Characterization of starch obtained from two different sources and methods

Analyses	Amaranth Starch (NaOH)	Amaranth Starch (NaHSO <sub>3</sub> )	Corn starch
Ashes	0.09 $\pm$ 0.001 <sup>a</sup>	0.05 $\pm$ 0.001 <sup>a</sup>	<0.12%
Starch content	91.5 $\pm$ 5.2% <sup>a</sup>	83.1 $\pm$ 0.2% <sup>b</sup>	92-96%
Protein	0% <sup>a</sup>	3.41 $\pm$ 0.19% <sup>b</sup>	<0.064% <sup>as</sup>
Reducing sugars	0.002 $\pm$ 0.1E-5% <sup>a</sup>	0.001 $\pm$ 0.2E-5% <sup>a</sup>	Traces
Color (L, a, b)	95.22 $\pm$ 0.459, 0.16 $\pm$ 0.014, 5.20 $\pm$ 0.166	91.43 $\pm$ 1.41, 0.68 $\pm$ 0.065, 8.53 $\pm$ 0.37	101.51 $\pm$ 0.653, 0.52 $\pm$ 0.038, 5.63 $\pm$ 0.101

It was observed a decrease close to 50% on *B. megaterium* growth time when amaranth

starch was used as unique carbon source during spread phase. Biggest production of  $\beta$ -CD was obtained on treatment at 60° C for 10 min. Kinetic parameters determined were  $\mu=13.188\pm0.12$ ,  $t_d=0.053\pm2E-03$ ,  $Y_{x/s}=0.307\pm1E-03$ . The major enzymatic activity and  $\beta$ -CD production were achieved at 38 h (Fig. 1).



**Fig.1** Kinetic of biomass, pH, CGTase activity of *B. megaterium* cultures with amaranth starch treated at a) 60°C and b) 90°C.

**Conclusion.** Amaranth starch treated at 60° C was a good substrate to produce CGTase. It might also be useful to obtain  $\beta$ -CD. This starch might be a different commodity that could be used instead of corn starch, which is generally used in the industry.

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