STUDIES ON BIO-UTILIZATION OF SOME AGRICULTURE WASTES TO PRODUCE α-AMYLASE ENZYME IMPORTANT IN FOOD PROCESSING

By

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A Thesis Submitted in partial Fulfillment
Of

The Requirements for the Degree of

DOCTOR OF PHILOSOPHY
in
Agricultural Sciences
(Food Science and Technology)

Department of Food Science
Faculty of Agriculture
Ain Shams University

2019

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ABSTRACT

Rania Menaaz Bayomy El-Feky: Studies on Bio-Utilization of Some Agriculture Wastes to Produce α-amylase Enzyme Important in Food Processing. Unpuplished ph.D Dissertation, Department Food Science, Faculty of Agriculture, Ain Shams University, 2019.

In the present study, optimization the production of fungal α-amylase by two different strains of fungi namely *Aspergillus* niger ATCC 102 and *Aspergillus oryzae* NRRL 6270 under solid state fermentation (SSF). Four different agro-industrial wastes e.g. Rice straw (*Oryza sativa*), Inner layers of sugarcane bagasse (*Saccharum officinarum*), Corn-gluten meal (CGM 1) (*Zea mays*) without addition and Corn-gluten meal after addition of corn steep liquor and Germ cake (CGM 2); which cause a serious environmental problem; were used to produce α-amylase. Chemical compositions of tested agro-industrial wastes were achieved and the results indicated that, an significant differences between fiber and protein content in current agro-industrial wastes. Different cultural conditions like moisture content of prepared agro-industrial by-products (30 to 70%), fermentation periods (12 to 120 h.) and incubation temperatures (25-35°C) were optimized to obtain the maximum yield of α-amylase activity. Process optimization for production of α-amylase was carried out in 250 ml Erlenmeyer flask and conducted using the previous substrates in a single parameter mode showing maximum enzyme activity. Among all the substrates inner layers of sugarcane bagasse was found to be best substrate for α-amylase production (4.62 U/g) in phosphate buffer as extracting medium for *Aspergillus oryzae* NRRL 6270. Amylase assay was performed by 3, 5 dinitrosalicylic acid (DNS) method with absorbance at 540 nm. Further, the suitable incubation period, moisture level and incubation temperature were examined. In case of *Aspergillus* niger ATCC 102, the optimized conditions for maximum

activity of α-amylase using rice straw, inner layers of sugarcane bagasse, Corn gluten meal without addition and corn gluten meal after addition recorded 2.47 U/g (solid substrate) at 40% moisture content after 12 hours of incubation at 30°C of incubation temperature, 4.23 U/g (solid substrate) at 60% moisture content after 72 hours of incubation at 30°C of incubation temperature, 1.99 U/g (solid substrate) at 70% moisture content after 72 hours of incubation at 30°C of incubation temperature and 1.49 U/g (solid substrate) at 30% moisture content after 48 hours of incubation at 30°C of incubation temperature, respectively. The results in case of *Aspergillus oryzae* NRRL 6270 recorded 3.80, 4.62, 1.92 and 3.09 U/g (solid substrate) for rice straw, inner layers of sugarcane bagasse, CGM 1 and CGM 2, respectively. The optimum conditions for SSF as follows temperature at 30°C. The enzyme was purified by ammonium sulphate precipitation. Partial purified α-amylase activity was determined. The partially purified enzyme optimally active at 30°C and pH 7.0 and the purified fungal α-amylase was used in bread making and compared with the commercial enzyme. The effect of addition of partial purified enzyme from different agro-industrial wastes on pan bread was achieved at two different concentrations 140 and 280 U. The addition of purified of α-amylase from inner layers of sugarcane bagasse at 140 U caused to increase the specific volume which was 6.3 and 6.1 for *Aspergillus* niger ATCC 102 and *Aspergillus oryzae* NRRL 6270, respectively. Textural study revealed an improved of pan bread quality, resulting in the decrease of hardness. Inner layers of sugarcane bagasse extracts that partial purified at 140 and 280 U decrease the value of hardness for *Aspergillus* niger ATCC 102 which was 70 N and 80 N, respectively. Corn gluten meal after addition of corn steep liquor and germ cake extract that partial purified at 280 U recorded 92 N for the same strain but for *Aspergillus oryzae* NRRL 6270 the extracts from inner layers of sugarcane bagasse at 140 and 280 U decrease the value of hardness which recorded 76 N and 86 N, respectively and the extract that partial purified from corn gluten meal after addition of corn steep liquor and germ cake was 92 N. The

sensory evaluation supported this result and confirmed the beneficial effect of addition on pan bread odor and crust color. The results showed a significant effect of the purified α-amylase in pan bread and allow us to improve the quality of the bread. The study indicated clearly that partial purified α-amylase is a potential candidate for future applications in bread making industry. Utilization of agro-industrial wastes provides an alternative method and value-addition in cost effectiveness of bioprocess. The obtained results demonstrated that, the potential application of the used strategy for α-amylase production from agro-industrial by-products.

Key words: Fungal alpha amylase; Agro-industrial by-products; Solid State Fermentation (SSF); UV- Spectrophotometer; amylase assay; Partial Purification; Dialysis bag; Application; pan bread.