IN SILICO STUDY OF ACRYLAMIDE TOXICITIES USING TOXTREE METHOD AND ITS ANALYSIS IN POTATO CHIPS USING HPLC METHOD

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INTRODUCTION

Acrylamide is among the contaminants commonly present in the food due to processing. Acrylamide is classified as a hazardous material which has potency as human's body carcinogenic for the case of cancer about 2% per year in the world. Acrylamide was found in food processed by the high temperature (above 120ºC) in food with high carbohydrates contents, such as potato chips.

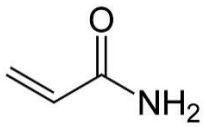

Figure 1. The chemical structure of acylamide

OBJECTIVES

The aim of this research was to obtain:

- the toxicities of acrylamide and the functional groups or moieties responsible for those toxicities using Toxtree software.
- analysis of acrylamide in potato chips using HPLC (High Performance Liquid Chromatography) method.

METHODS

Acrylamide toxicities were predicted using Toxtree v.2.5.1 software due to Benigni / Bossa rulebase and Structure alerts for the in vivo micronucleus assay in rodents as selected methods. Toxtree software also predicted the functional groups or moieties responsible for those toxicities. Some compounds belong to group 3 of IARC classification were applied as negative control. No positive control was necessary since acrylamide was group 2A of the IARC classification.

Potato chips samples preparation was done by eliminated the fats first and extracted using acetone. HPLC analysis performed by using column Shim-Pack VP-ODS 250Lx4,6 with PDA UV-Vis detector at a wavelength 197 nm, mobile phase acetonitrile : water (5 : 95), at flow rate of eluent 0.50 mL/min, the column temperature 28^{o}C.

RESULTS AND DISCUSSIONS

Acrylamide Toxicities Prediction Using Toxtree Software

The result of in silico toxicities study showed that acrylamide was both genotoxic carcinogenic and mutagenic, while isopropyl alcohol, phenol, and xylene as negative controls, showed neither genotoxic carcinogenic nor mutagenic effects. The α,β unsaturated double bonds formed vinylogic carbonyl group of acrylamide were responsible for those toxicities.

Table 2. Acrylamide toxicities prediction using toxtree software

Methods	Results
Benigni / Bossa rulebase for mutagenicity and carcinogenicity	- Structural alert for genotoxic carcinogenicity - Negative for nongenotoxic carcinogenicity
Structure Alerts for the in vivo micronucleus assay in rodents	- At least one positive structural alerts for the micronucleus assay

Potato Chips Analysis Using HPLC Method

Analysis of acrylamide in potato chips which sold in the Malang snacks shops showed that the retention time (Rt) acrylamide was around of 8.2 minutes. Calibration curve was performed in the range of 0.2-6.4 ppm and produced correlation coefficient 0.9996, Limit of Detection (LOD) 0.26 ppm and Limit of Quantitation (LOQ) 0.78 ppm. The results showed all samples provide retention time (Rt) equal to the standard acrylamide. But only 2 samples that give a pure peak belong to acrylamide. The levels of acrylamide in the samples were found 0.526 mg/kg and 0.644 mg/kg.

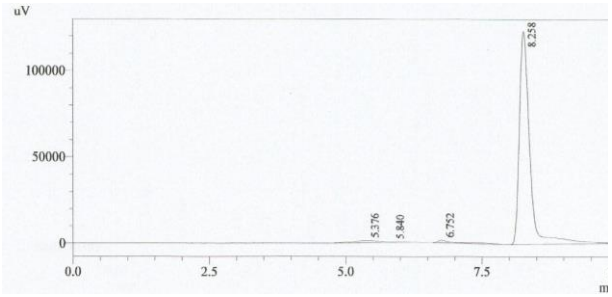

Figure 2. The chromatogram of optimized separation condition

The calibration curves for acrylamide using various concentrations of standards. The linearity range of the calibration curves was 0,2-6,4 mg/L and linear correlation was obtained 0.9996. The regression equation was y = 469758,5437x + 49905,0674. The Limit of Detection (LOD) of acrylamide was 0,26 ppm and Limit of Quantitation (LOQ) of acrylamide was 0,78 ppm.

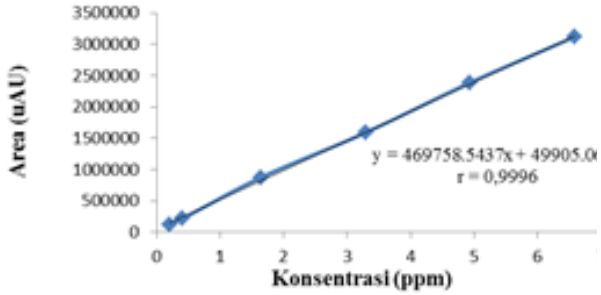

Figure 3. Linear correlation of acrylamide standards

The potato chips were analysed under the experimental conditions of the present HPLC method in three replicate. The results showed all samples provide retention time equal to the standard acrylamide. But only 2 samples that give a pure peak belong to acrylamide. It fullfilled the requirement of positive and pure containing acrylamide showed by retention time 8,2 minute, peak purity and match factor values more than 0,9000, λ maximum between 190-205 nm, and no impurity peaks. The levels of acrylamide in the samples were found 0.526 mg/kg and 0.644 mg/kg.

CONCLUSIONS

In silico toxicities study of acrylamide results were both genotoxic carcinogenic and mutagenic. The α,β unsaturated double bonds formed vinylogic carbonyl group of acrylamide were responsible for those toxicities. Analysis of acrylamide in potato chips which sold in the Malang snacks shops were found 0.526 mg/kg and 0.644 mg/kg.

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