STUDY OF THE FORMATION OF ACRYLAMIDE IN FRIED CASSAVA WITH COCONUT OIL, PALM OIL AND CORN OIL AS COOKING MEDIA

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ABSTRACT

Research on the study of acrylamide analysis using high performance liquid chromatography to maintain the accuracy and precision of this method has been carried out.. Lichrocart C-18 RP was used as the column with acetonitrile-water (5:95) pH 2.52 as the mobile phase. The time of retention was ± 7.3 minutes. Calibration curve showed a linear correlation between area under curve versus concentrations from 0.1 to 2 µg/mL with the correlation coefficient, r = 0.9999 and the equation of regression was y = 1973.9 + 21,8914 x. Limit of detection was 0.0125 µg/ml whereas limit of quantization was 0.0398 µg/ml. The optimum condition of analysis had the precision lower than 2% and average accuracy was between 99 to 101%. This method then was applied to study the formation of acrylamide in fried cassava with different cooking oils (coconut, palm and corn oils) as the media. The samples were extracted three times using dichloromethane-ethanol. Result showed that acrylamide was found in all samples which were 0.034 µg/g in coconut oil, 2.747 µg/g in palm oil and 0.41 µg/g in corn oil. The highest concentration of acrylamide was formed in palm oil.

I. INTRODUCTION

Swedish National Food Administration and Stockholm University reported the occurrence of acrylamide in various foods, especially in food which contained carbohydrate. The research showed that the formation of acrylamide mainly correlated with the heating process of the food at high temperature, such as potato chips, pop corn and biscuits (Otles, 2004).

Acrylamide ($CH_2=CHCONH_2$) forms as white crystalline compound. Its melting point is $84,5^0C \pm 0,3^0C$ whereas its boiling point is 125^0C (at 33,3 hPa). Its molecular weight is 71.08. Acrylamide dissolves in water (215.5 g/100ml), in acetone (63.1 g/100ml), and in ethanol 86.2 g/100ml) (Othmer, 1963).

The determination of acrylamide can be carried out using GC-MS and LC-MS (Othmer, 1963). High performance liquid chromatography is the most sensisitive method. It has several advantages such as the various kinds of detectors for various samples, the mobile phase can be used repeatedly, and the accuracy and precision of this method are high. (Mulja, 1995).

Several sources of hypothesis for the formation of acrolein are known. It may arise from degradation of amino acids and proteins, from degradation of carbohydrates, and from the Maillard reaction between amino acids or proteins and carbohydrates (3). Glycerol is degraded to acrolein, the unpleasant acrid black and irritating smoke, when the oil is heated above its smoke point (4). The smoke point is higher for oils with higher content of saturated fatty acids and lower content of polyunsaturated acids. The smoke points for some oils are as follows: palm 240° C, corn 160° C. Usually, the smoke starts to appear on the surface of heated oils before 175° C. The oil is first hydrolyzed into glycerol and fatty acids and then acrolein is produced by the elimination of water from glycerol by a heterolytic acid-catalyzed carbonium ion mechanism followed by oxidation (5).

$$CH_2(OH)-CH(OH)-CH_2(OH) \rightarrow$$
$$CH_2=CH-CHO$$

Glycerol   Acrolein
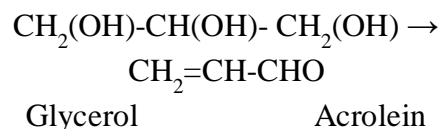
Acrolein can be converted into acrylamide by a series of fundamental reactions. However, both acrolein and acrylamide are reactive, because of their double bonds and the amino group of acrylamide. They can readily react further with other reactive groups present in the food matrix or formed during the heating process. For example, acrylamide can react with small reactive molecules, such as urea ($CO(NH_2)_2$) and formaldehyde (HCHO), or with glyoxal (($CHO)_2$), aldehydes (RCHO), amines (R_2NH), thiols (RSH) etc. Furthermore, the products shown in the following scheme can even react further in the same mode of reaction (5)

The aim of this research was to study the formation of acrylamide with different kind of vegetable oils as the cooking media. Cassava was chosen as the frying model of carbohydrate food.

II. Material and Methods
Materials
Chemicals. The following chemicals were obtained commercially : acrylamide *pro analysis* (99%, Merck), dichloromethane (Merck), ethanol grade HPLC (Merck), acetonitrile grade HPLC (Merck), phosphoric acid grade HPLC (Merck), aquabidest pro injection (Ikapharmindo), KBr *p.a* (Merck).coconut oil, palm oil, corn oil, and cassava were obtained from a local grocery store.

Instrument. HPLC: LC 10A-UV-vis SPD-10AV (Schimadzu), Vortex mixer 300, Ultrasonic shaker (NEY), Laboratory Shaker (IKA-HS 260), Spectrometry (Jena Specord 200), and pH meter.

The extraction method: The sample was weighed accurately about 15 grams then it was put into a flask shaker. About 60 mL of dichloromethane was added into the flask followed by 3 mL of ethanol then the mixture was shaken vigorously at 210 rpm for 50 minutes. The mixture was decanted. This procedure was repeated three times. The residue was washed with 20 mL of dichloromethane and was filtered A mixture of acetonitrile and water used as mobile phase was added to 30 mL of filtrate. The solvent was evaporated in water bath at 80^0C then the solution left was centrifuged at 4000 rpm for 30 minutes. The acrylamide was extracted in the acetonitrile layer.

Acrylamide standard solution: 262 mg of acrylamide was weighed and put into 250 mL volumetric flask. The acrylamide was dissolved with a mixture fo acetonitrile : water (5 : 95) pH 2.5 The concentration of acrylamide in this flask was 100 µg/mL.
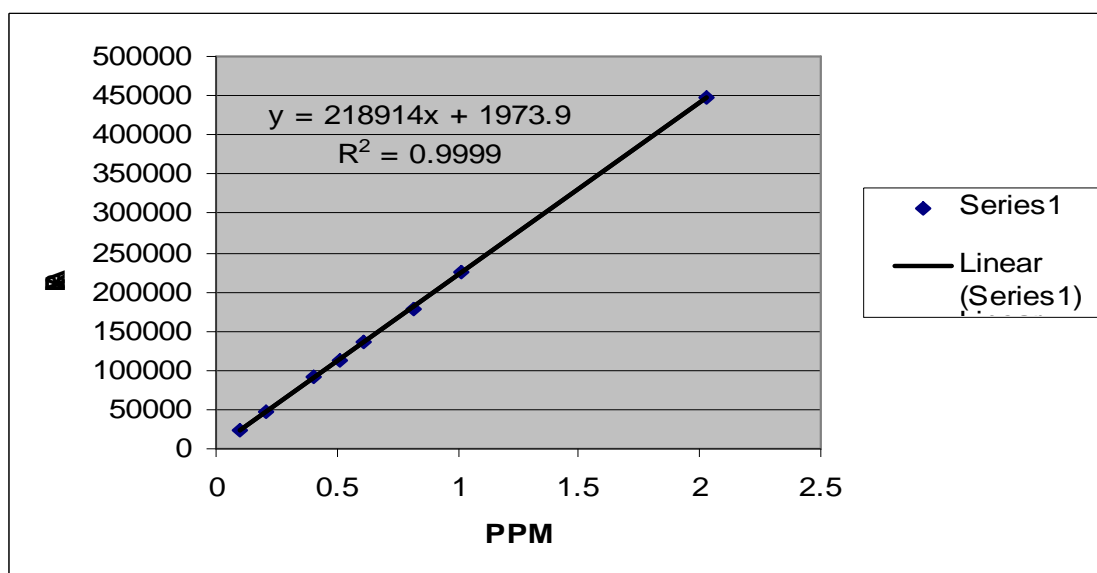
Calibration curve of acrylamide was obtained from acrylamide standard solution with concentrations 2; 1; 0,8; 0,6; 0,4; 0,2; 0,1 mg/L respectively.

Determination of acrylamide in the samples: The acrylamide extract was filtered through *Whatman* 0.45 µm and 20 µL of filtrate was injected to the HPLC column. The instrument used was Shimadzu SCL VP 10A equipped with Lichro CART RP-18 (125x4 mm internal diameter.), acetonitrile : water (5 :95) pH 2.5 as mobile phase, flow rate 0.5 mL/minutes, ultraviolet detector fixed at 210 nm. The volume injected was to the column was 20 µL.

Recovery. The fried cassava was weighed accurately about 15 grams and were soaked in 10 mL of acrylamide standard solution 2 mg/L for 12 hours The acrylamide in the samples was extracted with the same procedure as mentioned above. The acrylamide extract was filtered through *Whatman* 0.45 µm and 20 µL of filtrate was injected to the HPLC column.

III. RESULT AND DISCUSSION

The calibration curve of acrylamide was presented in Picture 1.

$$y = 218914x + 1973.9$$
$$R^2 = 0.9999$$

Picture 1. Calibration curve of acrylamide

The recoveries of acrylamide were presented as followed:

- 99,7217 ± 1,2153 %. at concentration 0,1016 µg/mL
- 99,2439 ± 1,8217 % at concentration 0,508 µg/mL
- 100,5914 ± 3,0576 % at concentration 1,016 µg/mL

LOD and LOQ of the method were obtained from calibration curve and the injection of mobile phase 8 times (Ibrahim S, 2001). The calibration curve was obtained from 8 concentrations of acrylamide standard solution within 0.1016-2.032 µg/mL of range. The

correlation coefficient *(r)* was 09999. LOD that was obtained from the measurement of blank signal was 0.0125 µg/mL and the LOQ was 0.0398 µg/mL.

The validated method was applied to study the formation of acrylamide in fried cassava using three different oils which were coconut oil, palm oil and corn oil.

The chromatogram of dichloromethane-ethanol extract of fried cassava with different cooking oils were presented in Picture 2, Picture 3 and Picture 4.
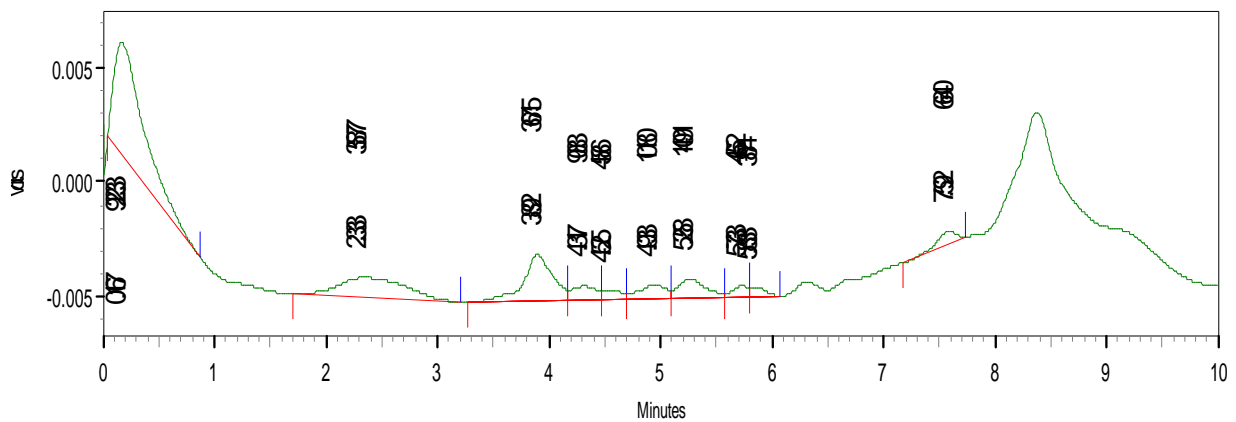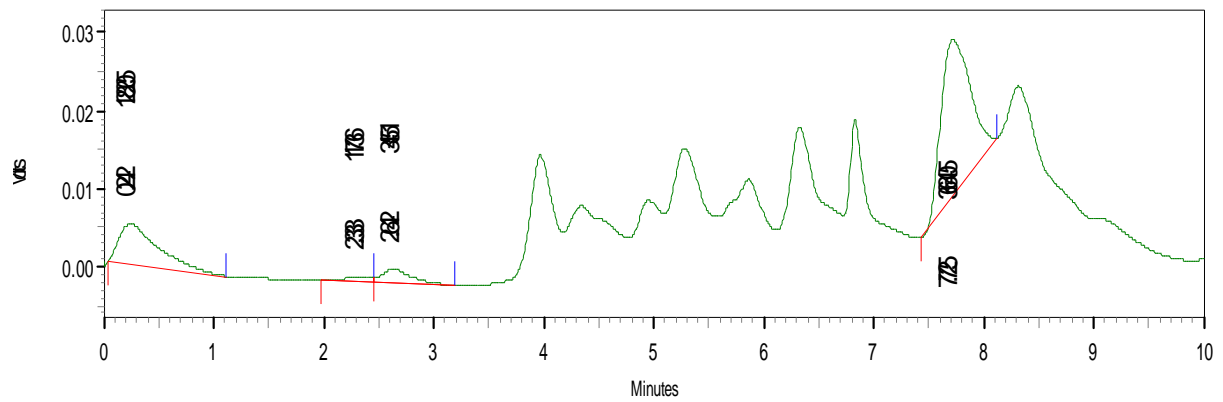

Picture 2. Chromatogram of dichloromethane-ethanol extract of fried cassava in coconut oil

The result of the determination of acrylamide formed in fried cassava with coconut oil as cooking media was presented in Table 1.

Table 1. The Result of the Determination of Acrylamide Formed in Fried Cassava with Coconut Oil as Cooking Media

Sample	Area	Concentration of acrylamide (µg/g)
A 1	6640	0.035
A 2	6438	0.033

Mean : 0.034 µg/g
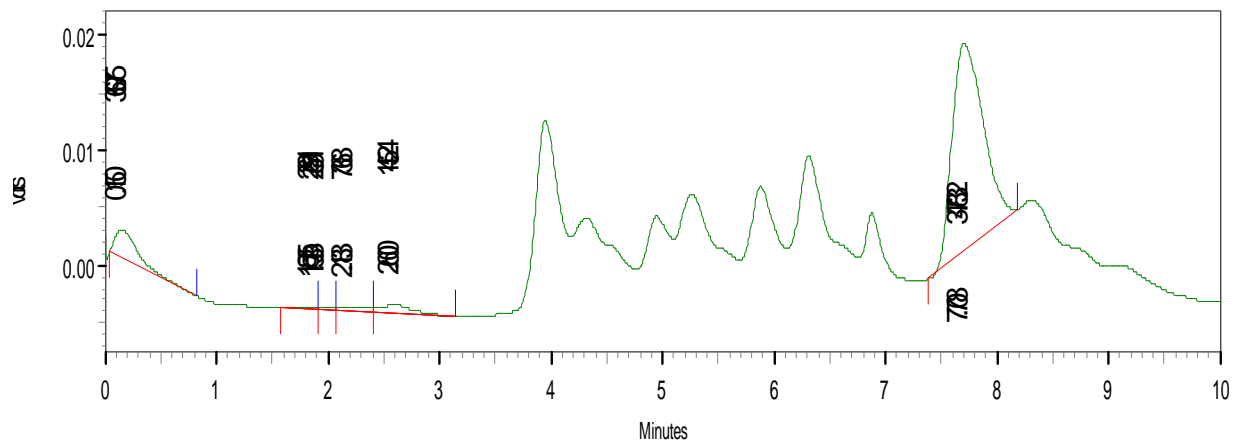Standard deviation : $1.414 . 10^{-3}$
Coefficient of variance : $0.021 . 10^{-3}$

Picture 3. Chromatogram of dichloromethane-ethanol extract of fried cassava in palm oil

The result of the determination of acrylamide formed in fried cassava with palm oil as cooking media was presented in Table 2.

Table 2. The Result of the Determination of Acrylamide Formed in Fried Cassava
with PalmOil as Cooking Media

Sample	Area	Concentration of acrylamide (µg/g)
B1	366405	2.774
B 2	352124	2.720

Mean   : 2.747 µg/g
Standard deviation   : 0.038
Coefficient of variance   : $1.05 . 10^{-5}$

Picture 4. Chromatogram of dichloromethane-ethanol extract of fried cassava in corn oil

The result of the determination of acrylamide formed in fried cassava with corn oil as cooking media was presented in Table 3.

Table 3. The Result of the Determination of Acrylamide Formed in Fried Cassava
with Corn Oil as Cooking Media

Sample	Area	Concentration of acrylamide (µg/g)
C1	56904	0.418
C2	52622	0.402

Mean   : 0.41 µg/g
Standard deviation   : 0.011
Coefficient of variance   : $2.01 . 10^{-5}$

IV. CONCLUSIONS

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Calibration curve showed a linear correlation between area under curve versus concentrations from 0.1 to 2 µg/mL with the correlation coefficient, r = 0.9999 and the equation of regression was y = 1973.9 + 21,8914 x. Limit of detection was 0.0125 µg/ml whereas limit of quantization was 0.0398 µg/ml. The optimum condition of analysis had the precision lower than 2% and average accuracy was between 99 to 101%. This method then was applied to study the formation of acrylamide in fried cassava with different cooking oils (coconut, palm and corn oils) as the media. The

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LITERATURES

Arthur, E.S., James, M.B., Roy, J.G., 1991. *Kromatografi.* Edisi II. Bandung : ITB.

Brandl F.[*], et. al,. *A rapid and convenient procedure for the determination of acrylamide in food stuffs.* 2002 :http : // ejeafche. Uvigo. Es / 1(3) 2002 /001132002F. Htm

Depkes R.I. 1979. *Farmakope Indonesia.* Edisi ketiga Jakarta: Departemen Kesehatan. Hal. 143-144.

Gritter. R.J., Bobbit, J.M., and Schwarting, A.E. 1991. *Pengantar Kromatografi.* Edisi kedua.Bandung : ITB. Hal. 186-230.

Ibrahim, S. 2001. *Pengembangan Metode Analisis Menggunakan Kromatografi Cair Kinerja Tinggi.* Seminar on HPLC Application for Analysis of Drugs, Food, and Environtment. Bandung : Dept. Farmasi F MIPA ITB. Hal. 1-19.

Johnson, E. L. dan R, Stevenson. 1991. *Dasar Kromatografi Cair.* Terjemahan Padmawinata. Bandung : Penerbit ITB. Hal. 1-54, 230-255, 300-301.

Mulja, M., dan Suharman. 1995. *Analisis Instrumen.* Surabaya: Airlangga University Press. Hal. 237-267.

Othmer, K. 1963. *Encyclopedia of Chemical Technology.* Vol 1. Second edition. New York: John Wiley and son, Inc. Page : 274-283.

Otles S. *Acrylamide in Food (chemical structure of acrylamide).* 2004: http://ejeafche.uvigo.es/3(5)2004/001352004.pdf.

Satiadarma K. 2004. *Asas Pengembangan Prosedur Analisis,* Edisi pertama. . Surabaya : Airlangga University Press. Hal. 46-51

Snyder, L. R., J, L, Glajch., and J, J, Kirkland. 1997. *Practical HPLC Method Development.* New York : John Willey & Sons. Page : 63-71; 76; 205-230; 292-314; 685-713.

Underwood A. L., et al. 1986. *Analisis Kimia Kuantitatif.* Penerjemah: Aloysius H.P. Jakarta: Elangga. Hal. 388-408

United State Pharmacopoeial Convention. 1997. *Drug Information for The Health Care Professional. vol. I, 17™ ed* Massachussets : United State Pharmacopoeial Convention Inc. Page : 791-793.