THE EFFECT OF USING COCONUT OIL, PALM OIL AND CORN OIL AS FRYING MEDIUM ON CONCENTRATION OF ACRYLAMIDE IN FERMENTED SOYBEANS PROCESSING*

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ABSTRACT

A research to identify and quantify acrylamide content in a cakes of fermented soya-beans with different three frying oil using reversed-phase high performance liquid chromatography (HPLC) method. The samples were prepared by cooking and baking above 120^{0}C fermented soybeansrature then extracted with dichloromethane-ethanol. The extracts were analyzed by HPLC, with condition as followed: C-18 column; acetonitrile-water (5:95) pH 2,52 mobile-phase; 0,5 ml/minute flow rate; and 210 nm wavelength. It was figured out that a fried fermented soybeans using corn oil contained 0,5778 µg/g acrylamide (8,202.10^{-3} standard deviation and 1,4195 % coefficient variation), using coconut oil 0,192 µg/g acrylamide (5,656.10^{-3} standard deviation 2,946 % coefficient variation), using palm oil 0,1455 µg/g acrylamide (6,081.10^{-3} standard deviation and 4,1794 % coefficient variation).

Introduction

The Swedish National Food Authority and the University of Stockholm have conducted valuable research in the field of food safety in April 2002. These researchers found microgram per kilogram to milligram per kilogram levels of acrylamide in foods (Tareke *et al*., 2002). Acrylamide is a reactive chemical, which is used as monomer in the synthesis of polyacrylamides used in purification of water, and in the formulation of grouting agents. Acrylamide is known as a component in tobacco smoke.

The International Agency for Research on Cancer (IARC) has classified acrylamide as probably carcinogenic to humans (Group 2A). Neurological effects have been observed in humans exposed to acrylamide. Properties, use and toxic effects of acrylamide are reviewed by IARC and EU (IARC, 1994).

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 (Mottram, 2002). Acrolein (Alarcon, 1976). Glycerol is degraded to acrolein, the unpleasant acrid black and irritating smoke, when oil is heated at fermented soybeansratures above the smoke point (26-29). The smoke point is higher

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for oils with higher content of saturated fatty acids and lower content of polyunsaturated acids. The smoke points for some of the main oils and fats are as follows: palm 240° C, peanut 220° C, olive: 210° C, lard and copra 180° C, sunflower and soybean 170° C, corn 160° C, margarine 150° C, and butter 110° C. Usually, the smoke starts to appear on the surface of heated oils before their fermented soybeansrature reaches 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 (Grivas *et al.*, 2004).

$$\underset{\text{Glycerol}}{CH_2(OH)\text{-}CH(OH)\text{-}CH_2(OH)} \rightarrow \underset{\text{Acrolein}}{CH_2\text{=}CH\text{-}CHO}$$

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 (Grivas *et al.*, 2002).

The aim of this research would be proofed of the influence of usage of coconut oil (with 84 % trygliseride), palm oil (with 84 % trygliseride) and corn oil (with 98,4 % trygliseride) as frying media to content of acrylamide at fermented soybeans.

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 oils, palm oils, corn oil, and fermented soybeans 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.

Methods

Preparation sample. The methods and procedure was adapted from Harahap *et al.* (2005). Fermented soybeans was fried until smoke point fermented soybeansrature by three frying media (coconut oils (sample A), palm oils (sample B), and corn oils (sample C)). The output of this process was called samples. 15 gram of samples were weighed, then it was dissolved in 60 mL dichloromethane and 3 ml ethanol ml, last be shacked with shaker laboratory at a speed at 210 rpm during 50 minute. The solution was filtered and filtrate was dissociated. The residue was cleaned by 20 mL dichloromethane, and then filtered. Into filtrate added 30 ml mobile phase, and it was evaporated in at 700 ^oC, furthermore be packed into centrifugation tube at 8000 rpm during 30 minute. The layer of mobile phase in centrifugation was taken into volumetric flask 25 ml and added mobile phase until border. The solution in the volumetric flask was filtered with membrane filter (0,45 millipore) and 20 μL filtrate was injected into HPLC instrument.

Analysis. The methods analysis adapted from Sanders *et al.* (2002) and Harahap *et* al. (2005). Samples were analyzed with a LC-10A (Schimadzu) interfaced to Detector UV-Vis SPD 10AV (λ = 210 nm). Column Lichro CART C-18 RP Select-B, 5 μm id. 4mm. Mobile phase: Acetonitrile: H_2O (5:95), 10 mM phosphoric acid, adjusted to pH 2.52. Flow rate: 0.5 mL/min. LC mode injection: Direct (no split). Injection volume: 20 μL.

Data Analysis. Response ratios (area of acrylamide in sample peak/area of acrylamide standard peak) were plotted against the corresponding concentration ratios for a series of five standards in dichloromethane. Standards contained concentrations ranging from 0 to 2 g/mL (0.1, 0.2, 0.4, 0.6, 0.8, 1 and 2). Linear regression resulted in a calibration curve from which concentration ratios in extracts were determined from measured response ratios.

Results and Discussion

Calibration curves

The equation of linear regression at curve calibrate which obtained from data processing of Table 1 was $y = 218914x + 1973{,}9$, with quadrates relation coefficient r^2 0,99. On the other hands, curve calibrate which obtained from this research have homogeneously of good data and small deviation.

Tabel 1. Data of calibration curves of acrylamide standard

Retention time (minutes)	Concentration (ppm)	Peak Area
7,317	0,10	23625
7,267	0,20	47372
7,733	0,40	92647
7,992	0,50	111997
7,342	0,60	135251
7,475	0,80	178659
7,572	1,00	224653
7,258	2,00	447120

The level of acrylamide of sample

The results of determined of acrylamide in sample can be seen in Table 2. In this research, the content of acrylamide in fried fermented soybeans with corn oil excess then content acrylamide in fried fermented soybeans with coconut oil and palm oil as frying medium. The average level of acrylamide in fried fermented soybeans with coconut oils, palm oils, and corn oils as frying medium were 0,5778 µg/g (SD $8,202.10^{-3}$ and coefficient variation 1,4195 %) 0,192 µg/g (SD $5,656.10^{-3}$ and coefficient variation 2,946 %), and 0,1455 µg/g (SD $6,081.10^{-3}$ and variation coefficient 4,1794 %), respectively.

Table 2. Identification and Quantification of acrylamide in fried fermented soybeans using different oils

Sample	Replication	Retention Time (minutes)	Concentration (µg/g)	Coeffiicient Variation
in	1	7,850	0,196	
Coconut oils	2	7,820	0,188	2,946 %
Average			0,1920 $\pm$ $5,7.10^{-3}$	
In	1	7,933	0,1498	
Palm oils	2	7,900	0,1412	4,1794 %
Average			0,1455 $\pm$ $4,08.10^{-3}$	
In	1	7,858	0,5720	
corn oils	2	7,850	0,5610	1,3730 %
			0,5665 $\pm$ $7,77.10^{-3}$	

The difference of content of acrylamide at fried fermented soybeans by using these three oils as frying medium was estimated to be caused by the existence of difference of fat or fatty acid composition from third oils used.

In the corn oil, the content of fatty acid unsaturated is dominant and that is linoleic acid (56%). On the other hand, it is predominated by trigilseride that is equal to 98 %. The formation of acrolein is known to increase with the increase in unsaturation in the oil and to lead to a lowering of the smoke point. The acrolein is higher for oils with higher content of trigliseride, because increasing of trigliseride in oils, increased content of glycerol that degradated to acrolein (Grivas *et al.*, 2004).

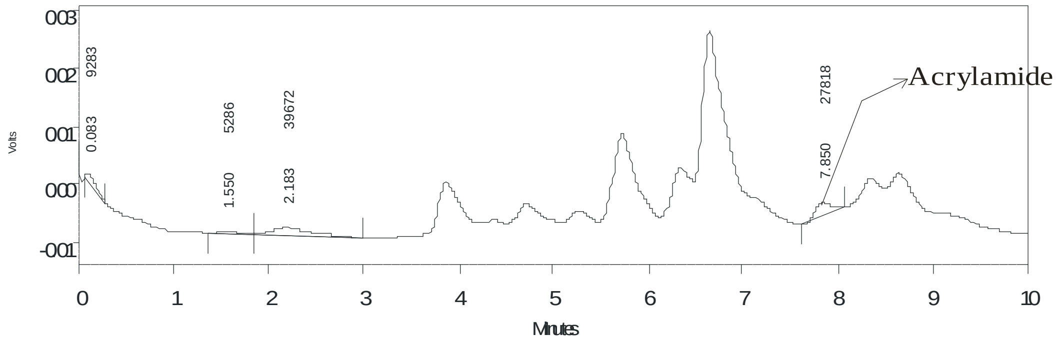

Figure 1. Chromatographic separation of the acrylamide in fried fermented soybeans with coconut oils as frying medium.

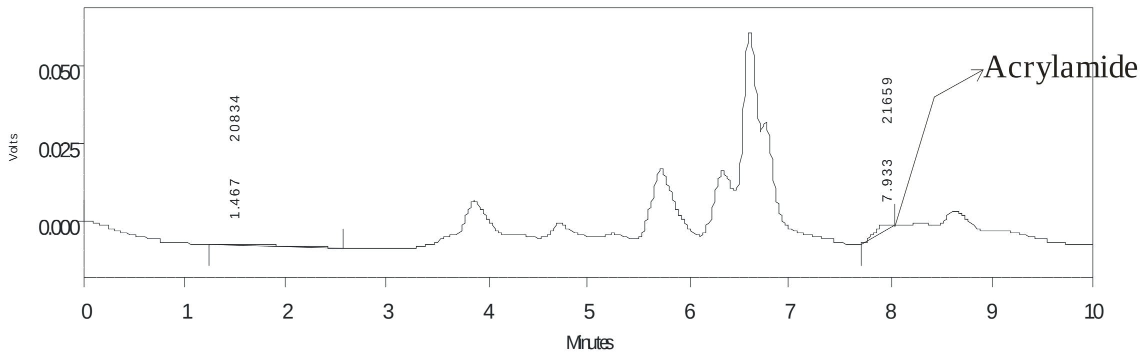

Figure 2. Chromatographic separation of the acrylamide in fried fermented soybeans with palm oils as frying medium.

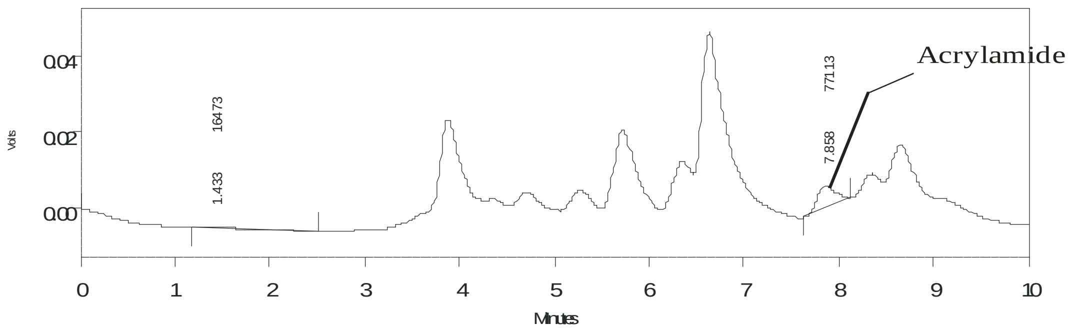

Figure 3. Chromatographic separation of the acrylamide in fried fermented soybeans with corn oils as frying medium.

Conclusion

In this research was known that be found microgram per kilogram levels of acrylamide in fried fermented soybeans using by different oils as frying medium. . The average level of acrylamide in fried fermented soybeans with coconut oils, palm oils, and corn oils as frying medium were 0,5778 µg/g (± $8,202.10^{-3}$ and coefficient variation 1,4195 %) 0,192 µg/g (± $5,656.10^{-3}$ and coefficient variation 2,946 %), and 0,1455 µg/g (± $6,081.10^{-3}$ and variation coefficient 4,1794 %), respectively.

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