



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 tempe.

## 2. Materials and Methods

### 2.1. Materials

**Chemicals.** The following chemicals were obtained commercially: acrylamide *pro analysis* (99%, Merck), dichloromethane (Merck), ethanol and methanol grade HPLC (Merck), acetonitrile grade HPLC (Merck), phosphoric acid grade HPLC (Merck), Cartridge C-18 for SPE (Solid Phase Extraction) from Waters, Aquabidest *pro injection* (Ikapharmindo), KBr *p.a* (Merck). coconut oils, palm oils, corn oil, and tempe 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.

### 2.2. Methods

#### Sample Preparation

Tempe was taken from Cileunyi Traditional Market in February 2006.

The methods and procedure was adapted from Harahap *et al.*[13]. Tempe was fried until smoke point temperature 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°C, 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. In this study, Solid Phase Extraction (C-18) was employed with methanol 60% as eluent for acrylamide extraction and clean-up[14,15].

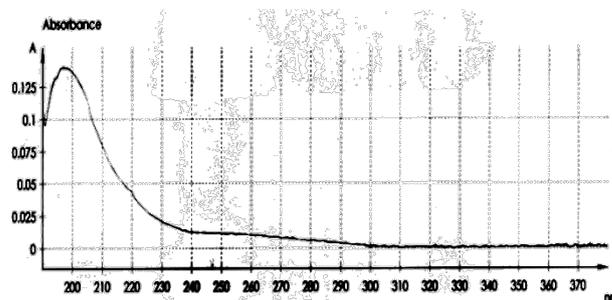
#### Determination of Maximum Wave Length of Acrylamide

Standard solution of acrylamide was produced with dissolved 262 mg acrylamide into 250 mL of mixture solution of mobile phase (acetonitrile: H<sub>2</sub>O (5:95) pH 2.52). This standard solution was diluted to obtain concentration of 4 mg/L. solution of 4 mg/L was determined by UV with range wave length 190-390 nm.

#### Calibration Curve

10 mg/L of solution standard was diluted to obtain range concentration 2.032, 1.016, 0.813, 0.609, 0.508, 0.406, 0.203, and 0.102 mg/L. The all concentration were performed by HPLC with detector UV in 198 nm. Concentrations were

plotted into area peak as shown in Fig. 1.



**Figure 1.** Maximum Absorbance of Acrylamide at concentration 0.508 µg/ml by Using Spectrometre (UV-1700 Pharmaspec-Shimadzu)

#### Repeatability Test

20 µl standard solution 10 mg/L was injected into column by using mobile phase and condition as mention above. This experiment was repeated three times, thus calculated variation coefficient.

#### Recovery Analysis

20 mg acrylamide was added into 20 gram fried tempe and crushed homogeneity. After that, 2 gram was taken from the sample and dissolved in solvent dichloromethane: ethanol (20:1). The mixture was shaken by laboratory shaker (IKA HS 260) in 30 0 minutes. Sample was filtrated and added 10 0 mM phosphoric acid.

#### HPLC Analysis

The methods analysis adapted from Sanders *et al.*[16] and Harahap *et al.*[13]. Samples were analyzed with a LC-10A (Schimadzu) interfaced to Detector UV-Vis SPD 10AV ( $\lambda = 210$  nm). Column Lichro CART C-18 RP Select-B, 5 µm id. 4mm. Mobile phase: Acetonitrile: H<sub>2</sub>O (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.

#### Analysis of Data

Response ratios area of acrylamide in sample peak/area of acrylamide standard peak was 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, 1.0, and 2.0). Linear regression resulted in a calibration curve from which concentration ratios in extracts were determined from measured response ratios.

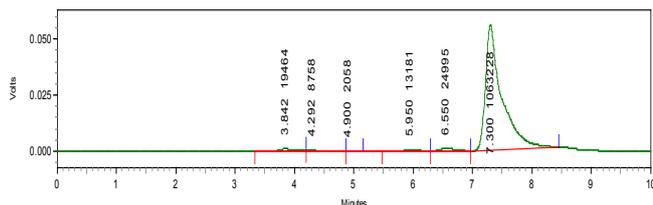
## 3. Results and Discussion

#### Validation of Analysis Methods

Acrylamide had maximum absorbance on wave length 198 nm that calculated at concentration 0.508 µg/ml into dichloromethane as solvent as shown in Fig 1. This wave length was applied to evaluated sample in detector of HPLC.

The various methods for determination of acrylamide includes about the occurrence, analytical methods, and extraction and clean-up procedures of acrylamide has been established, however special attention is given to chromatographic techniques applied for the occurrence and determination of acrylamide[17]. Here we studied by using HPLC

with addition standard methods. In this study, combination solvents was tried to get optimum analysis. The using of acetonitrile:water (5:95) in 10% phosphoric acid gave optimum condition in analysis. As shown in Fig. 2, peak of acrylamide in chromatogram was clear at 7.30 minutes.

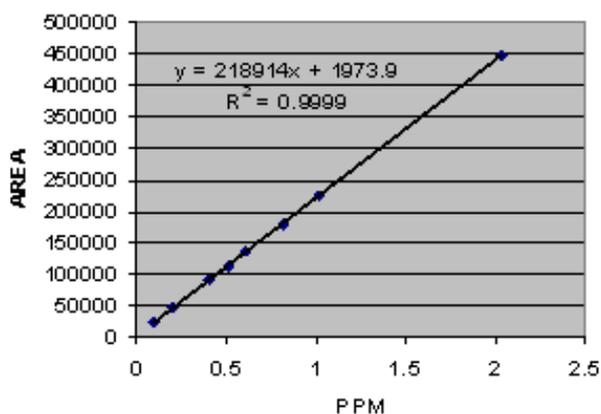


**Figure 2.** Chromatogram of Acrylamide use HPLC online UV detection with wave length 198 nm with mobile phase acetonitrile:water (5:95) and 10 mM phosphoric acid pH 2.52

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. The calibration curve of acrylamide was presented in Fig. 3.

**Table 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



**Figure 3.** Calibration curve of Acrylamide Analysis by HPLC in wave length 198 nm with mobile phase acetonitrile:water (5:95) and 10 mM phosphoric acid pH 2.52

In addition, calibration curve which obtained from this research have homogeneously of good data and small deviation. The recoveries of acrylamide were presented as followed:  $99.7217 \pm 1.2153\%$  at concentration  $0.1016 \mu\text{g/mL}$ ,  $99.2439 \pm 1.8217\%$  at concentration  $0.508 \mu\text{g/mL}$ ,  $100.5914 \pm 3.0576\%$  at concentration  $1.016 \mu\text{g/mL}$ .

LOD and LOQ of the method were obtained from calibration curve and the injection of mobile phase 8 times [18]. The calibration curve was obtained from eight concentrations of acrylamide standard solution within  $0.1016$ - $2.032 \mu\text{g/mL}$  of range. The correlation coefficient ( $r$ ) was 0.9999.

LOD that was obtained from the measurement of blank signal was  $0.0125 \mu\text{g/mL}$  and the LOQ was  $0.0398 \mu\text{g/mL}$ .

#### The level of acrylamide of sample

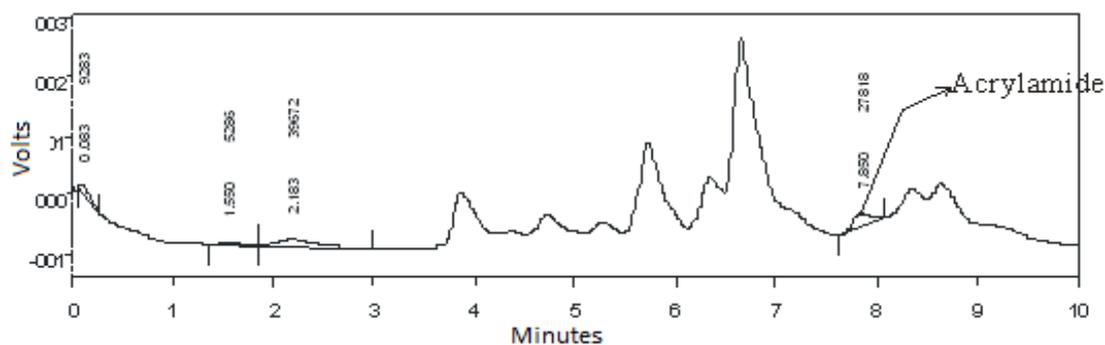
The results of determined of acrylamide in sample can be seen in Table 2 and Fig. 1-3. In this research, the content of acrylamide in fried tempe with corn oil excess than content acrylamide in fried tempe with coconut oil and palm oil as frying medium. The averages level of acrylamide in fried tempe with coconut oils, palm oils, and corn oils as frying medium were  $0.5778 \mu\text{g/g}$  (SD  $8.202 \cdot 10^{-3}$  and coefficient variation 1.4195%),  $0.192 \mu\text{g/g}$  (SD  $5.656 \cdot 10^{-3}$  and coefficient variation 2.946%), and  $0.1455 \mu\text{g/g}$  (SD  $6.081 \cdot 10^{-3}$  and variation coefficient 4.1794%), respectively. However, this content is far less than that found in French fries [10,15]. However, extraction process of acrylamide from crude sample of fried tempe play role in this analysis. Combining of dichloromethane and ethanol (20:1) was applied as solvent to attract acrylamide and then SPE with C-18 column was employed to isolate acrylamide in the mixture solution. This extraction method has been successfully to take acrylamide in the sample that indicated of recovery value in this analysis.

**Table 2.** Identification and Quantification of Acrylamide in Fried Tempe Using Different Oils

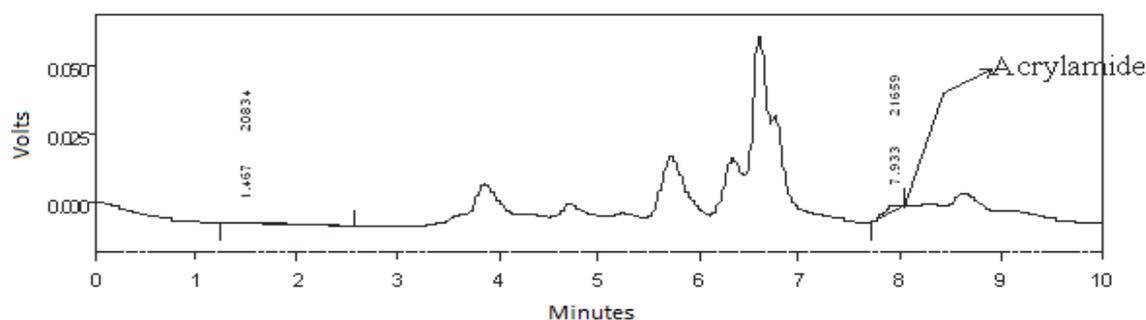
Sample	Replication	Retention Time (minutes)	Concentration ( $\mu\text{g/g}$ )	Coefficient Variation
<b>In Coconut oils</b>	1	7.850	0.196	
	2	7.820	0.188	
	3	7.850	0.188	2.946%
<i>Average</i>			$0.1920 \pm 5.7 \cdot 10^{-3}$	
<b>In Palm oils</b>	1	7.933	0.149	
	2	7.900	0.141	
	3	7.900	0.148	4.1794%
<i>Average</i>			$0.1455 \pm 4.08 \cdot 10^{-3}$	
<b>In corn oils</b>	1	7.858	0.5720	
	2	7.850	0.5610	
	3	7.850	0.5700	1.3730%
<i>Average</i>			$0.5665 \pm 7.77 \cdot 10^{-3}$	

Effect of oil as a medium frying medium in the formation of acrylamide has been published by previous research [19, 20]. The difference of content of acrylamide at fried tempe 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.

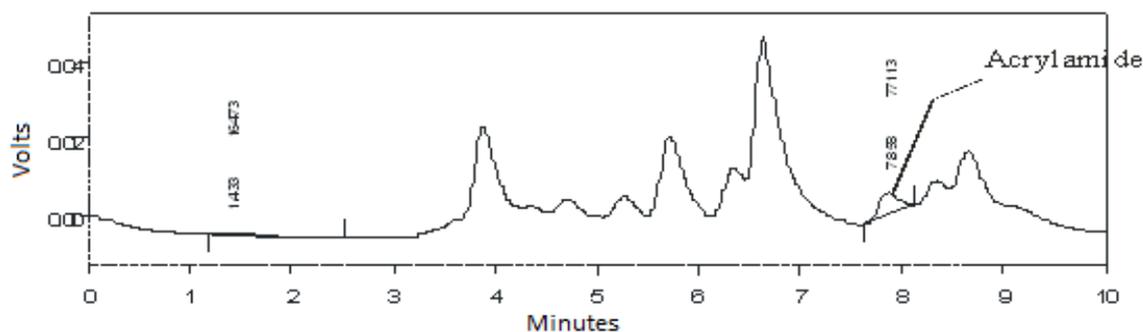
In the corn oil, the content of fatty acid unsaturated is dominant and that is linoleic acid (56%). On the other hand, it is predominated by trygliseride that is equal to 98%. The formation of acrolein is known to increase with the increase in unsaturation in the oil and to lead to a lowering of the smoke point. The acrolein is higher for oils with higher content of trygliseride, because increasing of trygliseride in oils, increased content of glycerol that degraded to acrolein [1].



**Figure 4.** Chromatographic separation of the acrylamide in fried tempe with coconut oils as frying medium in wave length 198 nm with mobile phase acetonitrile:water (5:95) and 10 mM phosphoric acid pH 2.52



**Figure 5.** Chromatographic separation of the acrylamide in fried tempe with palm oils as frying medium in wave length 198 nm with mobile phase acetonitrile:water (5:95) and 10 mM phosphoric acid pH 2.52



**Figure 6.** Chromatographic separation of the acrylamide in fried tempe with corn oils as frying medium in wave length 198 nm with mobile phase acetonitrile:water (5:95) and 10 mM phosphoric acid pH 2.52

## 4. Conclusions

In this study was known that found microgram per kilogram levels of acrylamide in fried tempe by using different oils as frying medium. The average level of acrylamide in fried tempe with coconut oils, palm oils, and corn oils as frying medium were  $0.5778 \mu\text{g/g}$  ( $\pm 8.202 \cdot 10^{-3}$  and coefficient variation 1.4195%)  $0.192 \mu\text{g/g}$  ( $\pm 5.656 \cdot 10^{-3}$  and coefficient variation 2.946%). and  $0.1455 \mu\text{g/g}$  ( $\pm 6.081 \cdot 10^{-3}$  and variation coefficient 4.1794%). respectively.

## ACKNOWLEDGEMENTS

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