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Determination of Acrylamide in Arabica Coffee from Six Production Regions of West Sumatra Using a Validated HPLC Method

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Abstract. *Acrylamide is a potentially carcinogenic compound formed during thermal processing through the Maillard reaction between asparagine and reducing sugars. Coffee roasting is one process that can lead to acrylamide formation. This study aims to determine acrylamide levels in Arabica coffee originating from six production regions in West Sumatra and evaluate the performance of a validated High-Performance Liquid Chromatography (HPLC) method for acrylamide analysis. Arabica coffee samples from Situjuah (A1), Lasi (A2), Marapi (A3), Pasaman (A4), Solok (A5), and South Solok (A6) were roasted, ground, extracted, and analyzed using HPLC equipped with a photodiode array detector. Method validation included assessment of linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). The calibration curve showed good linearity (R^2 = 0.9974). Precision and recovery values met acceptable analytical criteria. Acrylamide concentrations ranged from 4.3 to 12.1 µg/g among the coffee samples. Sample A5 showed the highest acrylamide concentration (12.1 µg/g), whereas sample A3 had the lowest (4.3 µg/g). Differences in acrylamide content were associated with coffee origin and bean characteristics. The validated HPLC method demonstrated satisfactory performance for acrylamide determination in coffee. These findings provide baseline information for food safety assessment and support optimization of roasting conditions to minimize acrylamide formation in Arabica coffee.*

Keywords: *Acrylamide; Arabica coffee; roasting conditions; HPLC validation; food safety*

Type of the Paper: Regular Article.

1. Introduction

Coffee is one of the most widely consumed beverages worldwide due to its distinctive flavor, aroma, and stimulating effects. Although coffee consumption has been associated with several health benefits, concerns remain regarding process-induced contaminants formed during roasting, particularly acrylamide. Acrylamide is an unsaturated amide compound generated during the thermal processing of carbohydrate-rich foods through the Maillard reaction between free asparagine and reducing sugars. This compound has attracted considerable attention because it is classified as a probable human carcinogen and has been associated with potential neurotoxic and genotoxic effects [1–5].

In coffee, acrylamide formation begins when roasting temperatures exceed approximately

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120 °C and increases substantially between 160 and 180 °C, where the Maillard reaction becomes more intensive. Acrylamide concentrations generally peak during the early to intermediate stages of roasting and subsequently decline at higher temperatures (>180–200 °C) due to thermal degradation and conversion into other reaction products [2,6–11]. Consequently, roasting conditions, particularly temperature and duration, play a critical role in determining coffee quality and acrylamide concentration [2,8,11].

The chemical composition of coffee beans, including carbohydrates, amino acids, proteins, and chlorogenic acids, strongly influences acrylamide formation during roasting. At the beginning of each roasting cycle, inserting green coffee beans into a roaster preheated to approximately 160 °C causes a rapid decrease in chamber temperature, followed by gradual heating as roasting progresses. During this process, complex chemical reactions occur, producing characteristic coffee flavor and aroma compounds, as well as acrylamide and other Maillard reaction products [8,12–15].

Although numerous studies have investigated acrylamide formation in coffee and the influence of roasting conditions, most have focused on commercial coffee products, industrial roasting systems, or mitigation strategies [2,6,7,10]. Information on acrylamide levels in Arabica coffee from different production regions in Indonesia, particularly West Sumatra, remains limited. Furthermore, studies combining acrylamide determination with comprehensive validation of High-Performance Liquid Chromatography (HPLC) methods for regional coffee products remain scarce. Therefore, this study provides the first comparative assessment of acrylamide levels in Arabica coffee originating from six major coffee-producing regions of West Sumatra using a validated HPLC method. The findings contribute to food safety evaluation, improve understanding of acrylamide occurrence in Indonesian Arabica coffee, and provide scientific information to optimize roasting practices, minimize acrylamide formation, and maintain coffee quality.

Initially, acrylamide is absent from raw materials of foods. However, it forms when a food ingredient is heated or thermally processed to a temperature of approximately 120 °C through broiling, baking, frying, or roasting [9]. Roasting is essential for developing these attributes, as it transforms raw green coffee beans through thermal processes to produce their characteristic taste, aroma, and colour [10].

Coffee aroma develops through a series of complex chemical reactions during roasting. However, these reactions may also produce undesirable compounds, including acrylamide. Acrylamide is primarily formed through the Maillard reaction between free asparagine and reducing sugars when coffee beans are heated at temperatures above approximately 120 °C, with formation increasing markedly between 160 and 180 °C during the early and intermediate stages of roasting [2,9,16,17]. Acrylamide concentration is influenced by several factors, including

roasting temperature, roasting duration, moisture content, pH, and the concentration of precursor compounds in the coffee beans [2,8,16]. Acrylamide is classified by the International Agency for Research on Cancer (IARC) as a Group 2A compound (probably carcinogenic to humans), and its occurrence in thermally processed foods has raised concerns regarding potential health risks associated with long-term dietary exposure [9,18]. Consequently, considerable research has focused on reducing acrylamide formation by selecting suitable coffee varieties, optimizing roasting conditions, and improving processing technologies, and maintaining the desired sensory quality of coffee [2,7,16,19]. Currently, regulatory agencies monitor acrylamide occurrence in foods because prolonged exposure may increase health risks. Therefore, mitigation strategies during food processing are strongly encouraged [9,18].

Green coffee beans contain approximately 300 volatile compounds and lack the color and characteristic flavor compared to roasted coffee [11]. They also contain non-volatile substances, such as carbohydrates, lipids, amino acids, and chlorogenic acids. There are also a lot of volatile chemical substances in coffee beans. The volatiles identified in roasted coffee beans include a large number of heterocyclic compounds such as pyrroles, furans, pyrazines, thiazoles, oxazoles, thiophenones, and imidazoles [12]. Many of these compounds are produced during roasting, including pyrolysis, caramelization, and the Maillard reaction [13]. The finished coffee's flavor and fragrance are also influenced by bean processing. Some factors are related to the raw material (green coffee selection and blending recipe), where others involve processing methods, including roasting, degassing and maturation, grinding, and percolation, all of which contribute to the final result [14]. These volatile compounds are identified by the human nose and influence our perception of coffee's taste. The coffee's fragrance is also influenced by the roast level [15].

The human nose can detect only approximately 5% of all volatile components, including pyrazines, furans, aldehydes, ketones, phenols, and sulfur compounds, which influence coffee flavor. The coffee's fragrance is also influenced by the roasting level [20]. Acrylamide forms during the early stages of roasting, but its concentration decreases as heating induces a chemical transition. Therefore, the amount of acrylamide generated is significantly influenced by the roasting rate. In 2017, the European Commission (Regulation (EU) 2017/2158) established guidelines outlining methods to reduce acrylamide in food and setting limits. Few studies have examined the optimal methods for altering roasting conditions in major coffee firms or cafés where coffee is roasted before serving. Because color, fragrance, and taste are crucial in determining coffee quality, roasting is a crucial step in coffee production.

To achieve the greatest taste and fragrance, roasting time and temperature should be carefully regulated. The type of coffee being manufactured and the type of roaster being utilized in real world scenarios affect the roasting conditions.

This study used the High-Performance Liquid Chromatography (HPLC) technique to determine the amount of acrylamide in Arabica ground coffee. This technique was selected because acrylamide might be hazardous and that roasting time and temperature are crucial. HPLC offers rapid analysis, effective compound separation, utilizes reusable liquid and a reusable column, accurate detection of trace substances, and high sensitivity [21].

2. Materials and Method

2.1. Material

The first stage of coffee production involves post-harvest processing, which is necessary to obtain green coffee beans. The beans are thoroughly cleaned using a technique known as wet processing [22]. In this procedure, the outer shell of the seeds is removed using a machine to chop and peel the red coffee cherries. The seeds are then fermented for a full day. After fermentation, they are thoroughly washed and sun-dried.

Six Arabica coffee samples were collected from six major coffee-producing regions in West Sumatra Province, Indonesia. The samples were coded according to their geographical origin as follows: A1 (Situjuah), A2 (Lasi), A3 (Marapi), A4 (Pasaman), A5 (Solok), and A6 (South Solok). The samples differed only on their geographical origin, not in different roasting temperatures or roasting times. To ensure a valid comparison, all samples underwent the same roasting conditions, grinding procedure, extraction method, and HPLC analytical protocol. Therefore, differences in acrylamide concentration among samples can be attributed primarily to variations in coffee origin and bean characteristics rather than processing conditions.

The Faculty of Pharmacy Laboratory at the University of Indonesia was used to analyze bioactive ingredients. The Service Laboratory Unit conducted additional testing, while Bogor Agricultural Institute performed calibration and certification. From April to June 2025, roasting was conducted at Fianda Coffee Roastery sites.

The materials used in this study included label paper, plastic, and dried Arabica coffee beans from West Sumatra Regency. Aquadest and water were utilized for the analysis. The equipment included a pH meter (Model PHS-3D-01, China), a color measuring device (Model HH06 Accu Probe, USA), roasting machines (Berto Coffee Roaster), a coffee bean grinder (Latina Grinder 600N), an analytical balance (Shimadzu Model, Japan), a 5 kg scale (OEM model, China), a desiccator, measuring cup, measuring flask, oven, coffee filter, weigh bottle, suction pipette, basin, spoon, and aluminum foil standing pouch (black, 250 g, 125 micron thick) for coffee packaging. Additional tools included camera, markers, and stationery.

The instruments used in this study are a set of High-Performance Liquid Chromatography (Shimadzu LC-6AD®), an ODS C18 column (250 × 4.6 mm) (Shimadzu Shimpack®), a

Photodiode-Array (PDA) detector (Shimadzu®), an analytical balance (Precisa XB 220A®), a laboratory shaker (Orbital shaker®), a Waterbath (Memmert®), measuring flasks (Iwaki®), beakers (Iwaki®), Erlenmeyer flasks (Iwaki®), measuring cups (Iwaki®), a 0.45 µm filter membrane, filter paper, vaporizer, aluminum foil, and research-supporting glassware.

The materials used for the analysis were six samples of ground West Sumatra Arabica coffee, acrylamide (C_3H_5NO) for synthesis (Merck), hexane (C_6H_{14}) (pa Merck), acetone (C_3H_6O) (pa Merck), acetonitrile (C_2H_3N) HPLC grade (Merck), and Aquabides Pro Injection (PT.Ikapharmindo Putramas).

2.2. Method

2.2.1. Sample Isolation

Six Arabica coffee samples were collected from six major coffee-producing regions in West Sumatra Province, Indonesia. The samples were coded according to their geographical origin as follows: A1 (Situjuah), A2 (Lasi), A3 (Marapi), A4 (Pasaman), A5 (Solok), and A6 (South Solok). All coffee beans underwent the same roasting, grinding, extraction, and analytical procedures to facilitate comparison of acrylamide levels among different production origins.

2.2.2. Preparation of Sample Test Solutions

2.2 g portions of ground coffee were measured for each sample. After adding 10 mL of hexane to remove fat, the mixture was agitated for five minutes at 350 r/min using an orbital shaker. The mixture was then allowed to settle until the solid components may sink to the bottom. To remove any remaining hexane, the liquid was gently drained off and the solid was permitted to dry. This fat removal procedure was conducted twice. Next, 20 mL of the fat-free coffee residue were combined with acetone and shaken once more on an orbital shaker set to 350 rpm for 20 minutes to obtain acrylamide.

After filtering the acetone portion using filter paper, the liquid was allowed to evaporate in a water bath. After mixing the remaining material with four milliliters of acetonitrile solution, water was added in a volumetric ratio of 15:85. After shaking the mixture to get rid of any leftover residue, filter paper was used once again. After passing through a 0.45 µm membrane filter, each sample solution was transferred into an HPLC vial. A 20 µL of each sample was injected into the HPLC system, and the area beneath the peak was determined under ideal analytical circumstances. Every measurement was performed in triplicate, and the area was noted. The regression equation from the calibration curve was used to determine the amounts of acrylamide [23].

2.2.3. Optimization of Mobile Phase and Analysis Conditions

Acetonitrile, distilled water, and methanol were combined at various volumetric ratios to create a mobile phase solution: 5:5:95, 100% distilled water, 10:90, 5:95, 2:98, and 15:85. A 20 µL of each mobile phase was then injected through a column containing an octadecyl silica

stationary phase, such as ODS or C18. Acrylamide must be present in the mobile phase at a standard concentration of 10 µg/mL. For the optimum separation, various flow rates (1.5 mL/min, 1 mL/min, and 0.5 mL/min) were tested, and the optimal combination of mobile phase and flow rate were selected. Performance was evaluated by examining the peak shape, height, and area, as well as the retention time of the standard acrylamide solution.

2.2.4. Acrylamide Identification

The chromatograms of the sample solution and the acrylamide standard solution were examined at the same retention time to qualitatively identify acrylamide. After filtration through a 0.45 µm filter, the test and the standard solution are placed in HPLC vials. The HPLC apparatus is then filled with 20 µL of each solution. The time required for each analyte to reach the detector (retention time) was recorded. If the test solution's time is the same or nearly the same as that of the standard solution, then acrylamide is present in the test solution.

2.3. Analysis Method Validation

2.3.1. Preparation of standards and acrylamide calibration curves

A 0.01-g portion of acrylamide standard was weighed and put in a 50 mL measuring flask to create a series of acrylamide solutions with varying concentrations. To get the desired concentration of 200 µg/mL, the acrylamide was dissolved in a solution of acetonitrile and water (15:85 by volume). Aliquots of this stock solution were then diluted with the same solvent mixture to prepare standard solutions at concentrations of 10, 20, 30, 40, and 50 µg/mL. For instance, fill a 5 mL flask with 0.25 mL, 0.5 mL, 0.75 mL, 1 mL, and 1.25 mL of the 200 microgram solution. Fill each flask to the brim with more mixture. Each solution was then filtered through a 0.45 µm membrane filter to filter each solution. Each solution should be injected into the HPLC system in 20 µL. To get the equation for the graph's straight line, $y = a + bx$, a calibration curve is created by measuring the area beneath each peak.

Acetonitrile, water, and methanol were combined at various volumetric ratios to create a mobile phase: 5:5:95, 100% water, 10:90, 5:95, 2:98, and 15:85. A 20 µL of the solution was injected into a mobile phase to pass through the column that contains an octadecyl silica stationary phase (often referred to as ODS or C18). To the mobile phase, add the standard acrylamide solution at a concentration of 10 µg/mL. Use 1.5, 1, or 0.5 milliliters per minute as the flow rate. The optimal mobile phase composition and flow rate were selected based on peak area, peak height, peak symmetry, and retention time. Using the same HPLC setup, the retention time of the chromatogram of the sample was compared with that of the acrylamide standard to determine if acrylamide is present in the sample.

Before being put into HPLC vials, the standard and sample solutions were filtered through a 0.45 µm filter. Each solution should be injected into the HPLC system in 20 µL. Acrylamide was

considered present in the sample if its retention time was identical or sufficiently close to that of the standard solution.

2.3.2. Linearity Test

The coefficient of determination (R^2), which indicates the linearity of the calibration curve, is obtained by analyzing the measurement data using the linear regression line equation. An R^2 value of not less than 0.99 was considered indicative of good linearity.

2.3.3. Testing of Detection Limits and Quantities

The straight lines from the calibration curves are used to determine the quantitation and detection limits. The LOD was calculated as three times the residual standard deviation divided by the slope, whereas the LOQ was calculated as ten times the residual standard deviation divided by the slope. The residual standard deviation (Sy/x) used as the basis for the response's standard deviation. These computations used the line's slope from the equation $y = a + bx$.

2.3.4. Repeatability Precision Test

The sample was measured six times at a concentration of 30 µg/mL of acrylamide standard solution in order to perform the precision test. This test verifies the precision of the findings. The RSD number, or relative standard deviation, is used to quantify precision, with an RSD value of 1% or less are acceptable.

2.3.5. Accuracy Test (% Recovery)

A simulation technique known as spiking was used to conduct the recovery test. This entails mixing a sample solution devoid of acrylamide with a few standard acrylamide solutions. 10 milligrams was added to 2.2 grams of ground coffee as the standard solution. This was conducted three times using the same extraction procedure used to prepare the sample solution. A percentage representing the recovery value from the reference standard was then computed. It was discovered that the acceptable recovery rate ranged from 85–110%.

2.4. Application of Acrylamide Levels by HPLC

Using a pipette, 0.5 mL of each test solution was added to a volumetric flask and diluted with the mobile phase to reach a total volume of 5 mL. Each solution was then filtered through a 0.45 µm filter membrane and, under the proper analytical conditions, injected up to 20 µL into the HPLC apparatus. The peak area was measured, and a calibration curve equation was used to determine the amount of acrylamide in the sample. This was conducted for 12.5 minutes at a wavelength of 200 nm and at a flow rate of around 0.5 mL/min. A Shimadzu Shim Pack octadecylsilica (ODS or C18), which measured 250 mm × 4.6 mm was used as the stationary phase. The mobile phase consisted of acetonitrile and distilled water at a 15:85 (volume to volume) ratio. Under ideal analytical circumstances, the area under the curve (AUC) was calculated. Three attempts were made at this measurement. The regression equation derived from the calibration

curve was used to determine the acrylamide content.

3. Results and Discussion

This study demonstrates that the level of acrylamide in roasted coffee beans can vary. The amount of acrylamide produced depends on the roasting temperature and duration. Roasting is crucial because it enhances the flavor and aroma that consumers find appealing [24]. The criteria commonly used to evaluate the quality of green coffee includes seed size, colour, shape, processing method, crop year, cup quality, and presence of defective seeds and of strange matters [13]. The beans are roasted in a drum set to a temperature of around 160°C. Depending on the heat applied, the temperature decreases to 100–105°C after a minute before rising again. Heat from the drum causes the water within the beans to evaporate. The beans turn brown due to a chemical reaction that occurs when the water evaporates. A color measuring device (Accu Probe HH06) measures the beans' brightness. All of the physical changes during roasting occur simultaneously and influence one another. These changes can be measured using instruments or observed immediately, for instance, by observing the size and color of the beans. The brightness (L) number indicates the amount of light of a certain color that the beans reflect. Brighter beans have a higher L value. The beans' color shift indicates the completion of roasting. Acrylamide formed in coffee beans during roasting was partially transferred into brewed coffee [20].

When the temperature reaches 160 and 175°C and moisture level is really low, the Maillard process begins to intensify [11]. states that green beans include over 300 volatile chemicals, while [12] notes that they also contain non-volatile substances such as amino acids, lipids, carbohydrates, chlorogenic acids, and other volatile organic compounds. Furans, pyrazines, ketones, pyrolysis products, phenols, hydrocarbons, acids, anhydrides, aldehydes, esters, alcohols, sulfur compounds, and more are the primary chemicals present in green beans. According to Toco and Farah [13], many of these compounds are produced by the pyrolysis, caramelization, and Maillard reaction of non-volatile substances in green beans. The Maillard process produces hundreds of distinct chemicals that give coffee its distinct flavor and aroma. The Maillard reaction, which occurs when amino groups from asparagine interact with carbonyl groups from reducing sugars during heating, is the primary pathway for acrylamide (prop-2-enamide, AA) formation in food [20].

At a standard concentration of 10 µg/mL, the maximum absorption of acrylamide may be determined using a UV spectrophotometer at a wavelength of 196–198 nm. Nevertheless, measuring at this wavelength range may result in excessive solvent absorption and impurity interference, making it challenging to select the appropriate mobile phase, particularly because ground coffee has a complicated composition [25]. High background noise and an unstable baseline were derived from testing at 190 nm [26]. Therefore, the study examined acrylamide at

200 nm, which produces superior outcomes (no influence from solvents) [9].

Previous HPLC research on acrylamide in instant coffee and ground coffee (tubruk) revealed 5.71 ± 0.03 µg/g in instant coffee and 7.03 ± 0.001 µg/g in ground coffee (tubruk) [27]. Similarly, research on civet coffee and conventional ground coffee revealed that each sample's acrylamide levels exceeded the WHO's acceptable limit.

According to the Food and Drug Administration (FDA), consumers can still safely consume ground or instant coffee as long as they don't consume more than 16 grams per day. According to the World Health Organization (WHO), 0.3 to 0.8 milligrams of acrylamide per kilogram of body weight per day is the acceptable limit that individuals can consume through food. Considering an average adult weighing around 60 kg, the acceptable daily intake of acrylamide is between 18 and 48 micrograms. Adults typically consume one to three cups of coffee per day. One cup of coffee may contain 640 to 7305 micrograms of acrylamide if one spoonful of ground coffee weighs around 5 grams [28].

According to these figures, individuals should avoid consuming more than 15 grams of acrylamide per day, or 1920–21915 micrograms, of coffee. The amount of acrylamide in coffee is significantly influenced by roasting speed. Compared to dark-roasted coffee, light-roasted coffee has much higher acrylamide [9].

Acrylamide levels in ground coffee are influenced by several factors [2,7,29]. One explanation is that coffee contains molecules called acrylamide precursors, which are converted to acrylamide during roasting. The amount of acrylamide produced after roasting might vary depending on the kind of coffee since these precursors are derived from amino acids and reducing sugars. Additionally, the amount of acrylamide produced depends on the roasting temperature and duration. Acrylamide production increases with roasting speed and temperature decrease [14,21].

3.1. Optimization of Mobile Phase and Analysis Conditions

The first step in this investigation is determining the ideal conditions for clean separation using High Performance Liquid Chromatography (HPLC). It examines the effects of altering the chromatogram's shape, flow rate, and mobile phase on the acrylamide standard solution. The mobile phase consisted of distilled water and acetonitrile at a volume ratio of 15:85, with a detection wavelength of 200 nm and a flow rate of 0.5 mL/min. The acrylamide peaks appeared at 6.7 minutes with this configuration. Because there was only one peak visible in the comparison of the sample and standard chromatograms, this combination was selected. This indicates that the chromatogram is symmetrical, the baseline is stable and straight, the separation is good, and there are no overlapping peaks. This enables precise measurement of the peak area with minimal interference.

3.2. Acrylamide Identification

Acrylamide was identified by comparing the retention time of the sample peaks with that of the acrylamide standard solution (10 µg/mL). The standard chromatogram exhibited a distinct peak at a retention time of 6.791 min. Similar peaks were observed in all Arabica coffee samples with retention times ranging from 6.712 to 6.781 min (Table 1), indicating acrylamide in the samples. The small variation in retention times (< 0.1 min) was within the acceptable range for chromatographic analysis and confirmed the identity of acrylamide.

Representative chromatograms of the acrylamide standard and selected coffee samples are presented in Fig. 1. The absence of significant interfering peaks near the acrylamide retention time demonstrated adequate selectivity of the analytical method.

Table 1. Retention times of the acrylamide standard and the analyzed coffee samples.

Coffee	Sample	Retention time (t_R)
Standard	Acrylamide standard 10 µg/mL	6.791
A1	Situjuah	6.762
A2	Lasi	6.766
A3	Marapi	6.712
A4	Pasaman	6.769
A5	Solok	6.781
A6	South Solok	6.739

The acrylamide standard exhibited a retention time of 6.791 minutes. Comparable peaks were observed in all coffee samples with retention times ranging from 6.712 to 6.781 minutes, confirming the presence of acrylamide. Chromatographic conditions were as follows: C18 column (250 × 4.6 mm, 5 µm), distilled water–acetonitrile (15:85, v/v) as the mobile phase, flow rate of 0.5 mL min^{-1}, PDA detection at 200 nm, and injection volume of 20 µL.

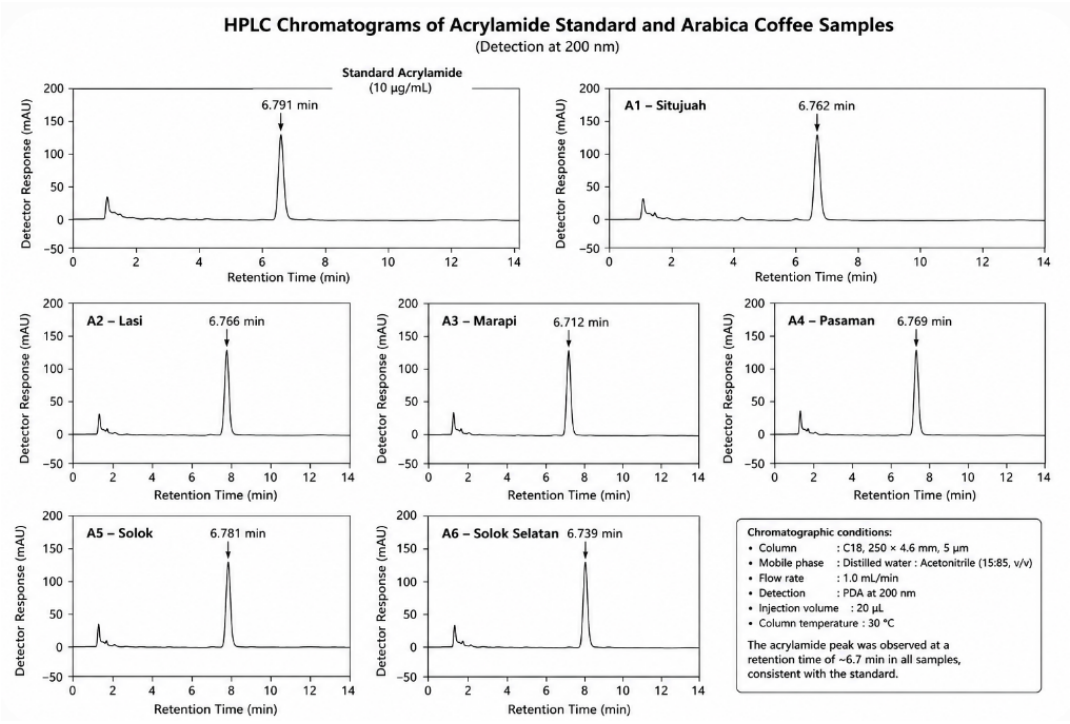


Fig. 1. HPLC chromatograms of acrylamide standard (10 µg/mL) and Arabica coffee samples (A1-A6).

Fig. 1 presents the HPLC chromatograms of the acrylamide standard and Arabica coffee samples. The acrylamide standard showed a well-defined peak at a retention time of 6.791 minutes. Similar peaks were detected in all analyzed coffee samples with retention times ranging from 6.712 to 6.781 minutes. The close agreement between the retention times of the samples and the standard confirmed the presence of acrylamide in the analyzed coffee samples. No significant interfering peaks were observed near the acrylamide retention time, indicating adequate selectivity of the chromatographic method. These results demonstrate that the validated HPLC method is suitable for acrylamide determination in Arabica coffee samples.

3.3. Analysis Method Validation

The validation test demonstrates that acrylamide may be examined by the HPLC technique utilizing distilled water and acetonitrile as the mobile phases in a 15:85 (by volume) ratio. The technique is precise, produces reliable findings, and can handle various compounds being tested without any difficult to identify problems. Linearity, detection and quantification limits, accuracy, and precision are among the validation factors that are examined.

3.4. Linearity Test

The linearity of the HPLC method was evaluated using acrylamide standard solutions at concentrations of 10, 20, 30, 40, and 50 µg/mL. Each standard solution was analyzed at a detection wavelength of 200 nm, and the corresponding peak areas were recorded. A calibration curve was constructed by plotting peak area (y) against acrylamide concentration (x).

As shown in Fig. 2, the detector response increased proportionally with increasing acrylamide concentration, indicating a strong positive relationship between analyte concentration and chromatographic response. Linear regression analysis yielded the calibration equation $y = 27002x + 32606$, with a coefficient of determination ($R^2 = 0.9974$). The high R^2 value demonstrates excellent linearity within the studied concentration range and confirms that the detector response was directly proportional to acrylamide concentration.

Linearity is an important validation parameter that evaluates the ability of an analytical method to produce responses proportional to analyte concentration. The R^2 value exceeded 0.99, indicating that the HPLC method exhibited excellent linear performance and is suitable for the quantitative determination of acrylamide in coffee samples. The calibration data and regression curve are presented in Table 2 and Fig. 2, respectively.

Table 2. Calibration data of acrylamide standard solutions (10–50 µg/mL).

No.	Acrylamide Concentration (µg/mL)	Retention Time (t_R)	Peak Area (Area Units)
1	10	6.791	322566
2	20	6.768	569388
3	30	6.778	810289
4	40	6.794	1107365
5	50	6.765	1403656

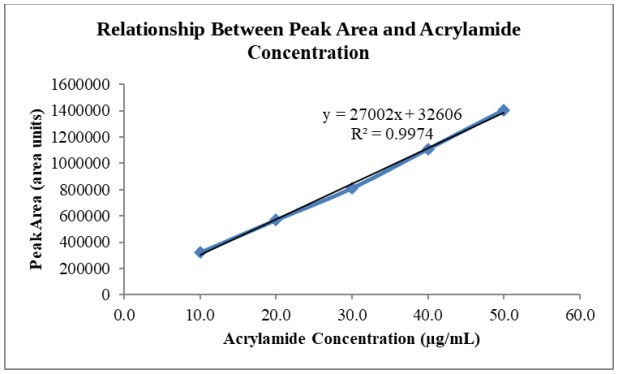


Fig. 2. A graph of the relationship between area and acrylamide concentration.

3.5. Detection Limits (LOD) and Quantity Limits (LOQ)

Determining the acrylamide detection limit and quantitation limit is essential for evaluating the performance of analytical methods. Although it may not always be precisely determined, the detection limit (LOD) is the lowest concentration of the material that may be discovered in a sample. The LOQ is the lowest concentration that can be quantified with acceptable accuracy and precision. The concentration must exceed the detection and quantitation limits to yield reliable findings. The regression equation and the standard deviation can be used to compute these limitations. The detection limit (LOD) and the quantitation limit (LOQ) for acrylamide, calculated from the calibration curve, were 2.8 µg/mL and 9.4 µg/mL, respectively.

3.6. Precision Test

Precision refers to the degree of agreement among repeated measurements of the same sample. Standard deviation (SD) and relative standard deviation (% RSD) are used to quantify precision. Repeatability, also known as repetition, examines the precision of results obtained from the same experiment conducted under the same circumstances, such as the same subject, apparatus, setting, and time. The test was conducted six times at a concentration of 30 µg/mL to ensure repeatability. The relative standard deviation (%RSD) was 0.246%, which is less than 1%, according to the precision test findings at 30 µg/mL.

3.7. Accuracy Test (% Recovery)

Accuracy refers to the closeness of an analysis's findings to match the actual concentration of the material under examination. The amount of the additional material recovered is used to measure it. Using a simulation technique, a known quantity of acrylamide was added to a coffee sample to verify accuracy. After analysis, the amount of acrylamide in the sample was contrasted with the amount that was added. It was prepared in the same manner as a typical sample, with 2.2 g of ground coffee containing 10 mg of acrylamide. The HPLC system received 20 µL of each sample, and three measurements were performed. The amount of the additional standard discovered was indicated by the recovery percentage. Table 3 shows that the average recovery rate

for ground coffee was 99%. These findings satisfied the analytical method's validation requirements, which call for a recovery rate of 85–110%.

Table 3. Acrylamide accuracy (% recovery) test data in ground coffee samples.

Sample	Area	Acrylamide levels after standard addition (mg)	% acrylamide recovery
A1	74288378	9.913	99%
A2	75373857	9.9483	99%
A3	72191747	9.9389	99%
A4	78373746	9.9125	99%
A5	78383648	9.9149	99%
A6	79972774	9.9212	99%

3.8. Determination of Acrylamide Levels

Acrylamide levels in six samples of ground coffee are shown in Fig. 3. All samples were roasted under the same conditions (see Section 2.1), so the differences in acrylamide content among the six samples (4.3–12.1 µg/g) are attributed to differences in coffee origin and bean characteristics, such as the content of precursors (free asparagine and reducing sugars), rather than to roasting conditions. Sample A5 (Solok) had the highest acrylamide content, whereas sample A3 (Marapi) had the lowest. Other studies indicate that acrylamide levels rise at the beginning of roasting, peak at a particular temperature, and then rapidly decline [30]. Because roasting temperature and duration were not varied in this study, their effects on acrylamide formation were not evaluated. Taste and art are both involved in roasting, and both can affect the finished sensory experience.[,,][]

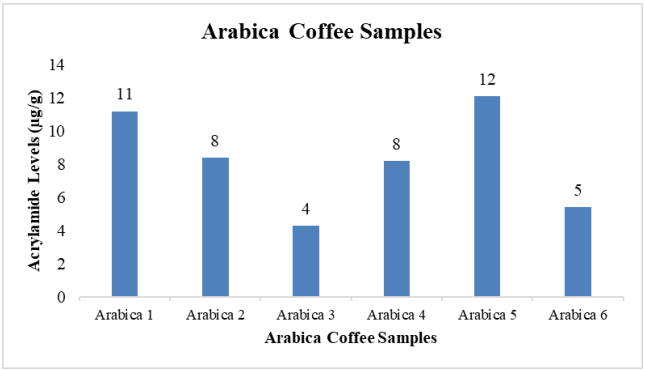


Fig. 3. Acrylamide content of Arabica coffee samples from six production regions in West Sumatra.

More than 900 distinct compounds are produced at the end of the roasting process, and the way the coffee is ground and processed also affects its ultimate flavor [14].

However, only around 5% of these substances, such as sulfur compounds, pyrazine, furans, aldehydes, ketones, and phenols can be detected by the human nose and influence taste. The degree of roasting can alter the coffee's flavor and aroma [15]. Since the early 2000s, foods have contained the solid, crystalline chemical acrylamide [16,17]. Acrylamide production and partial breakdown during industrial coffee roasting follow a similar pattern to small-scale or laboratory tests. Acrylamide reaches its maximum temperature of 175°C during industrial roasting, after which it

rapidly decreases as temperatures climb. Acrylamide levels in medium-roasted coffee, which is roasted to temperatures close to 215°C, are lower than the European Commission's requirement. Consequently, it's critical to monitor the amounts of acrylamide, particularly in medium-roasted coffee.

The levels of essential components such as asparagine, reducing sugars, and sucrose were monitored throughout the industrial coffee roasting process. The results demonstrate that heating sucrose can also produce acrylamide and asparagine. This finding aligned with studies conducted between 2000 and 2002 [16,17]. The estimated safe daily intake of acrylamide is 2.60 parts per billion of acrylamide per kilogram of body weight. The safe limit for a 70 kg person is 182 parts per billion. Based on the acrylamide content per cup, an individual should not consume more than eighteen cups of coffee in a day if one cup has that much acrylamide. To enhance quality and flavor, West Sumatra produces arabica coffee by carefully controlling temperature and fermentation time. This also involves developing roasting techniques that produce coffee with fewer acrylamide and more beneficial chemicals [31]. Since acrylamide may be hazardous to humans and may influence the neurological system, it is crucial to eliminate it.

4. Conclusions

A validated HPLC method was successfully applied to determine acrylamide in Arabica coffee originating from six production regions in West Sumatra. The analytical method demonstrated acceptable performance in terms of linearity, precision, accuracy, LOD, and LOQ. Acrylamide concentrations ranged from 4.3 to 12.1 µg/g across the analyzed samples. The highest concentration was observed in sample A5 (Solok), whereas the lowest was found in sample A3 (Marapi). The results indicate that differences in acrylamide content among samples are associated with coffee origin and intrinsic characteristics of the coffee beans. This study provides baseline data on acrylamide occurrence in West Sumatran Arabica coffee and supports the application of validated HPLC methods for food safety monitoring. Future studies should investigate the effects of roasting temperature, roasting duration, and precursor compounds on acrylamide formation and mitigation.

Abbreviations

PDA	Photodiode Array
HPLC	High Performance Liquid Chromatography
LOD	Limit Of Detection
LOQ	Limit Of Quantitation
FDA	Food and Drug Administration
WHO	World Health Organization
RSD	Relative standard deviation

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available because they form part of an ongoing research project.

Credit Authorship Contribution Statement

Rince Alfia Fadri, Henny Fitri Yanti: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Writing - Original Draft Preparation. **Mutia Elida, Evawati, Yenni Muchrid, Irwan Roza :** Supervision, Validation, Methodology, Writing Review and Editing. **Muhammad Isa Dwijatmoko, Mulianti:** Formal Analysis, Visualization, Resources, Data Interpretation, Writing – Review and Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Use of AI in the Writing Process

The authors used Claude (Anthropic) during the preparation of this manuscript to reorganize the in-text citation order and reference list and to format the revised manuscript in response to the editorial comments. After using the tool, the authors carefully reviewed and edited the content and take full responsibility for the content of the publication.

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