Journal of
Food and Pharmaceutical Sciences

Original Article

Evaluation of Acrylamide Levels in Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*) Coffee Roasting Variations for Nutraceutical Capsules

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Received: 1 October 2025; Revised: 17 December 2025; Accepted: 4 March 2026; Published: 25 April 2026

Abstract: Coffee roasting is an essential process that determines the flavor and aroma of coffee, yet it also contributes to the formation of acrylamide, a carcinogenic compound. Acrylamide is produced as a by-product of the Maillard reaction, which occurs between reducing sugars and amino acids at high roasting temperatures. This study aimed to evaluate acrylamide content in Arabica coffee (*Coffea arabica*) and Robusta coffee (*Coffea canephora*) under different roasting levels, and to assess its safety for nutraceutical capsule formulation. The experimental procedure included roasting beans at three levels (light, medium, and dark). Acrylamide analysis was performed using UV-Vis spectrophotometry with the standard addition method. Data were analyzed using Kruskal–Wallis and Mann–Whitney tests with JASP software. Results showed that acrylamide levels increased with increasing roasting time in both Arabica and Robusta coffee. The lowest acrylamide concentrations were observed at 10 minutes of roasting, particularly in the n-hexane fraction measuring 72.1154 μg/kg in Arabica coffee and 109.6825 μg/kg in Robusta coffee, while the highest levels were detected in the water fraction at 20 minutes of roasting, reaching 299.8834 μg/kg in Arabica coffee and 400.5265 μg/kg in Robusta coffee. Statistical analysis confirmed that roasting variations significantly affected acrylamide formation ($p < 0.05$), whereas no significant difference was found between coffee types ($p > 0.05$). Although the n-hexane fraction yielded the lowest acrylamide content, ethyl acetate at 10 minutes of roasting was identified as the optimal condition, as it produced relatively low acrylamide levels (124.1045 μg/kg in Arabica and 136.8083 μg/kg in Robusta) while preserving bioactive compounds suitable for nutraceutical formulation. Evaluation of granule quality and capsule characteristics indicated that all parameters met standard requirements. In conclusion, nutraceutical capsules can be formulated from the ethyl acetate fraction of coffee roasted for 10 minutes, ensuring safety and functional benefits.

Keywords: acrylamide, arabica and robusta coffee, nutraceutical capsules, roasting variations

1. INTRODUCTION

Coffee has become one of the most widely consumed beverages and an integral part of modern lifestyle. It belongs to the botanical family Rubiaceae, which comprises around 500 genera and more than 6,000 species. The genus *Coffea* consists of slightly more than 100 species, yet only two are widely marketed, namely *Coffea arabica* (approximately 70%) and *Coffea canephora* (around 30%). Limited supplies also come from *Coffea liberica* and *Coffea excelsa*, each contributing less than 1% [1].

The high consumption of coffee has increased attention toward its processing, particularly during the roasting stage. The roasting process plays a crucial role in shaping the main characteristics of coffee, such as aroma, flavor, and color. However, the high temperatures applied in this stage may trigger the formation of thermal contaminants that are both toxic and carcinogenic, notably acrylamide. This compound is formed at elevated temperatures (≥120°C) through the Maillard reaction between reducing carbohydrates and amino acids, especially asparagine [2].

This molecule was first identified in 1994 as a "possible human carcinogen" by the International Agency for Research on Cancer (IARC). Later, in 2002, the Swedish National Food Authority confirmed its presence in many carbohydrate-rich food products [3]. Human exposure to acrylamide can occur through various pathways, including the consumption of food and beverages. Health risks associated with prolonged acrylamide exposure include neurotoxicity [4].

Based on research by Esposito [5] reported that acrylamide levels in Arabica and Robusta coffee beans range between 154–495 μg/kg. However, these variations are strongly influenced by roasting conditions, coffee variety, and the analytical methods applied. Therefore, the analysis of acrylamide in coffee poses challenges due to the complexity of the sample matrix, which can affect measurement accuracy. Acrylamide is a byproduct of the coffee roasting process, and its occurrence is relatively low. The complex coffee matrix, rich in bioactive compounds, may interfere with the accuracy of acrylamide analysis. Hence, the standard addition method is employed, as it can reduce matrix effects, improve accuracy, and validate the presence of the target compound in the sample [6].

Despite the presence of contaminant compounds such as acrylamide, coffee remains rich in bioactive constituents, including caffeine, chlorogenic acid, ferulic acid, and caffeic acid, which provide various health benefits [7]. These properties highlight the potential of coffee as a raw material for nutraceutical products, particularly in capsule form, offering practical use, enhanced stability, and standardized dosing.

However, most previous studies on acrylamide in coffee have focused on roasted beans or brewed beverages for conventional consumption, while investigations addressing acrylamide levels in relation to nutraceutical capsule development remain limited. Moreover, systematic evaluations of acrylamide content in Arabica and Robusta coffee across specific roasting variations relevant to nutraceutical formulation are still scarce. Therefore, this study aims to evaluate and compare acrylamide levels in Arabica and Robusta coffee subjected to different roasting variations using the standard addition method, as a basis for selecting safe and functional raw materials for coffee-based nutraceutical capsules. This study integrates food safety assessment with functional product development, providing new insights into optimal roasting conditions that minimize acrylamide formation while preserving the health-promoting potential of coffee bioactive compounds.

2. MATERIALS AND METHODS

2.1. Place and time of research

This study was carried out at the Pharmacy Laboratory, Universitas Telogorejo Semarang, between February and August 2025.

2.2. Chemical and reagents

Ethanol 96% (C_2H_6O), *n*-hexane (C_6H_{14}), ethyl acetate ($C_4H_8O_2$), aquadest (H_2O), acrylamide p.a. (Sigma Aldrich), avicel 101, PVP, aerosil, magnesium stearate, talc, and *amylum maydis*.

2.3. Tools and material

UV-Vis spectrophotometer (Agilent Cary 60), 10 mL volumetric flask (Iwaki), 25 mL volumetric flask (Iwaki), 50 mL volumetric flask (Iwaki), beaker glass (Iwaki), measuring cylinder, volumetric pipette (Iwaki), dropper pipette, stirring rod, mortar, pestle, spatula, porcelain crucible, disintegration tester, analytical balance, flowmeter, caliper, oven, and sieves with mesh sizes 14 and 20.

2.4. Sampling and plant determination

The Arabica and Robusta coffee samples were sourced from Jl. Raya Kalitangi, RT.02/RW.09, Dlimar, Genting, Jambu District, Ambarawa, Central Java. Plant identification was conducted at the Center for Research and Development of Medicinal Plants and Traditional Medicines, Tawangmangu, Central Java to confirm the authenticity of the coffee bean plants used in the research.

2.5. Preparation of Arabica and Robusta Coffee Beans

Arabica and Robusta coffee cherries are processed using natural methods. Green coffee beans, harvested three months after their uniform red color were dried in the sun until dry, then separated from the skin. Coffee fruit was separated between coffee beans and coffee peel using a wet sorting

method by washing the coffee beans with tap water to minimize dirt attached to the Robusta coffee beans and air-dried, then ground with a pulping machine to separate the pulp of coffee beans. After that, the Arabica and Robusta coffee beans were sorted by selected coffee beans. The selected coffee beans met the criteria that were not defective. The coffee beans were then ground with a grinder into powder to reduce the particle size and then filtered with a 60-mesh sieve. A total of 2.5 kg of green Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*) coffee beans were roasted at 200 °C for varying times of 10, 15, and 20 minutes.

2.6. Coffee extraction and fractionation

A total of 500 g of Arabica and Robusta coffee powder, roasted at 200 °C for 10, 15, and 20 minutes, was macerated with 96% ethanol at a ratio of 1:10. The maceration was carried out for 3 × 24 hours with stirring every 6 hours, followed by filtration to obtain the macerate. The residue was then re-macerated with 96% ethanol (1:5) for 24 hours, filtered, and the filtrates were combined. The combined filtrates were evaporated using a rotary evaporator at 45 °C and further concentrated in a water bath at 50 °C until a thick ethanolic extract was obtained [8]. After obtaining the thick extract, 10 g of the ethanolic extract was dissolved in 100 mL of hot water (70 °C) and subsequently fractionated successively using a separatory funnel with n-hexane, ethyl acetate, and water at a ratio of 1:1.5 [9].

2.7. Extract standardization

2.7.1 Moisture content

Moisture content was determined using a moisture balance. A total of 1 g of extract was weighed in an aluminum dish and then placed in the instrument at 105 °C [10].

2.7.2 Ash content

The thick extract was placed into a pre-ignited and tared silica crucible, then leveled evenly. The sample was incinerated gradually in a furnace at 600 °C until all carbon residues were completely removed. The crucible was cooled and weighed to determine the total ash content[11]:

2.8. Determination of Acrylamide Content by UV-Vis Spectrophotometry

2.8.1 Determination of Acrylamide Levels in Samples

A total of 25 mg of the extract, n-hexane fraction, ethyl acetate fraction, and aqueous fraction of Arabica and Robusta coffee was each dissolved in 25 mL of distilled water. From this solution, 0.5 mL was taken and diluted to a final volume of 25 mL. The resulting solution was then distributed into six volumetric flasks, each containing 4 mL. Then, the standard was added to the six flasks with a concentration of 10 ppm as much as 0; 2; 2.5; 3; 3.5; and 4 mL.

2.8.2. Preparation of Acrylamide stock solution

A total of 50 mg of acrylamide standard was weighed and dissolved in 50 mL of distilled water. From this solution, 0.5 mL was taken and diluted with distilled water to a final volume of 50 mL.

2.8.3. Determination of Maximum Wavelength

A total of 4 mL of a 10 ppm standard solution was diluted with distilled water to a final volume of 10 mL. The absorbance was then measured using a UV spectrophotometer within the wavelength range of 190–400 nm.

2.8.4. Calibration Curve Determination

Aliquots of 2, 2.5, 3, 3.5, and 4 mL of a 10 ppm standard solution were each diluted with distilled water to a final volume of 10 mL. The absorbance of each solution was measured at the maximum wavelength (λmax). Calculate the absorbance values using the standard curve equation $y = bx + a$.

2.8.5. Precision Determination

Precision was evaluated using measurement data at the standard addition level of 3 mL, performed in three replications on the ethyl acetate fraction samples of Arabica and Robusta coffee with a roasting time of 10 minutes. The obtained concentration values were calculated as %RSD to

assess the repeatability at this level. Precision was determined by calculating the %RSD using the following equation [12]:

$$\text{RSD}\ (\%) = \frac{\text{SD}}{\bar{X}} \times 100\% \quad \text{..... (1)}$$

Description:
SD : Standard Deviation; $\bar{x}$: Mean concentration

2.8.6. Accuracy Determination

Accuracy was determined using the standard addition method, in which standard solutions of 2, 2.5, 3, 3.5, and 4 ppm were added to the sample matrix of the ethyl acetate fractions of Arabica and Robusta coffee with a roasting time of 10 minutes. Accuracy was determined by calculating the %Recovery using the following equation [12]:

$$\%\text{Recovery} = \frac{C_1 - C_2}{C_3} \times 100\% \quad \text{..... (2)}$$

Description :
C_1 : Concentration of the sample matrix with added standard
C_2 : Concentration of the sample matrix without added standard
C_3 : Concentration of the standard added to the sample matrix

2.8.7 Determination of LOD and LOQ

The LOD and LOQ values were determined using the following equations:

LOD = 3.3 × Sy/X ..... (3)

LOQ = 10 × Sy/X ..... (4)

Description :
Sy/x : Standard Deviation; b : Slope

2.9. Formulation of Nutraceutical capsule

Table 1. Formulation of Nutraceutical capsule [13][14][15]

Ingredients	Formulation		Function
	F1	F2	
EA fraction of Arabica coffee beans	25 mg	-	Active ingredient
EA fraction of Robusta coffee beans	-	25 mg	Active ingredient
Avicel 101	2.5%	2.5%	Disintegrant
Polyvinyl-pyrrolidone (PVP)	2%	2%	Binder
Aerosil	3%	3%	Adsorbent
Mg. Stearate	1%	1%	Lubricant
Talc	2%	2%	Glidant
Amylum maydis	ad 150 mg	ad 150 mg	Diluent

Note: EA=Ethyl Acetate

2.9.1 Preparation of nutraceutical capsule

The extract fractions of Arabica and Robusta coffee beans were mixed with powdered excipients until homogeneous. Polyvinylpyrrolidone (PVP) was dissolved in distilled water at a concentration of 20% b/v. The 20% b/v PVP solution was then added to the mixture to form wet granules, which were subsequently sieved through a mesh 14 screen and dried at 40 °C for 15 minutes. The dried granules were further sieved using a mesh 20 screen [16]. In the lubrication stage, magnesium stearate and talc were passed through a 20-mesh sieve, weighed, and mixed. After the blending process, magnesium stearate and talc were added. Once all components were homogeneously mixed, the mixture was filled into capsule shells using a capsule filling device [17].

2.10. Evaluation of nutraceutical capsules

2.10.1. Granule moisture content

A total of 1 g of granules was placed into a moisture balance to determine the moisture content. The granules were considered acceptable if the moisture level was within the range of 2–5% [18].

2.10.2. Flow rate

A total of 10 g of granules was weighed and placed into a funnel with a closed outlet. The outlet was then opened, and the flow time of the granules was measured using a stopwatch. The flowability of the granules was considered good if the time required to discharge 10 g of granules did not exceed 1 second [13].

2.10.3. Angle of Repose

A total of 10 g of dried granules was weighed, then placed into a funnel with a closed outlet. The granules were poured and leveled at the surface. The height and the radius of the base of the granule pile formed were then measured [13].

2.10.4. Compressibility

A total of 100 mL of granules was placed into a graduated cylinder of a Density Tester. The compressibility index was considered to indicate good compressibility if it was within the range of 11–15% [13].

2.10.5. Content Uniformity

The content uniformity test was carried out by randomly selecting not fewer than 30 units. The content of 10 individual units was determined and weighed. The acceptance value was then calculated [19].

2.10.6. Disintegration time

The disintegration test was performed on 6 capsules using a disintegration tester, with the temperature maintained at 36–38 °C. The capsules were considered compliant if the disintegration time was ≤ 30 minutes [20].

2.11. Data Analysis Methods

The dependent data variable was acrylamide levels, which were influenced by independent variables of different roasting variations and coffee types with statistical methods using JASP software version 0.95. The data were analyzed using the non-parametric test. The Kruskal–Wallis test (as the non-parametric form of ANOVA) was used to evaluate differences in roasting variations, and the Mann–Whitney U test (as the non-parametric form of the t-test) was used to compare coffee types.

3. RESULTS AND DISCUSSION

3.1. Determination of Arabica and Robusta Coffee

Determination of Arabica and Robusta coffee samples was carried out at the Center for Research and Development of Medicinal Plants and Traditional Medicines at B2P2TOOT, Tawangmangu, Central Java with letter numbers TL.02.04/D.XI.6/6937.219/2025 and TL.02.04/D.XI.6/6937.220/2025. The results of plant identification showed Arabica coffee beans with another name *Coffea arabica* L. and Robusta coffee beans with another name *Coffea canephora* Pierre ex A. Froehner.

3.2. Extract standardization

3.2.1. Moisture content and Ash Content

Roasting is a processing method known to be effective in reducing the moisture content of food materials [21]. Shown in Figure 1, there is an inverse relationship between roasting time and moisture content, indicating that increasing roasting duration resulted in a significant reduction in moisture levels. This finding is consistent with the results reported by Correa et al. [22], who stated that longer roasting times lead to greater water loss due to evaporation. In addition, extended roasting increases heat transfer to the coffee beans, thereby accelerating water evaporation and significantly reducing their moisture content.

The determination of total ash content aims to provide an overview of the mineral content, both internal and external, originating from the initial processing stages until the formation of the simplicia. A high ash content value may indicate the presence of inorganic contaminants or heavy metals resistant to high temperatures. The results of the total ash content determination in the samples met the requirements set by the Indonesian Herbal Pharmacopoeia (FHI), which is less than 10%. Therefore, the tested coffee extract can be considered to meet quality standards based on the total ash content parameter [23].

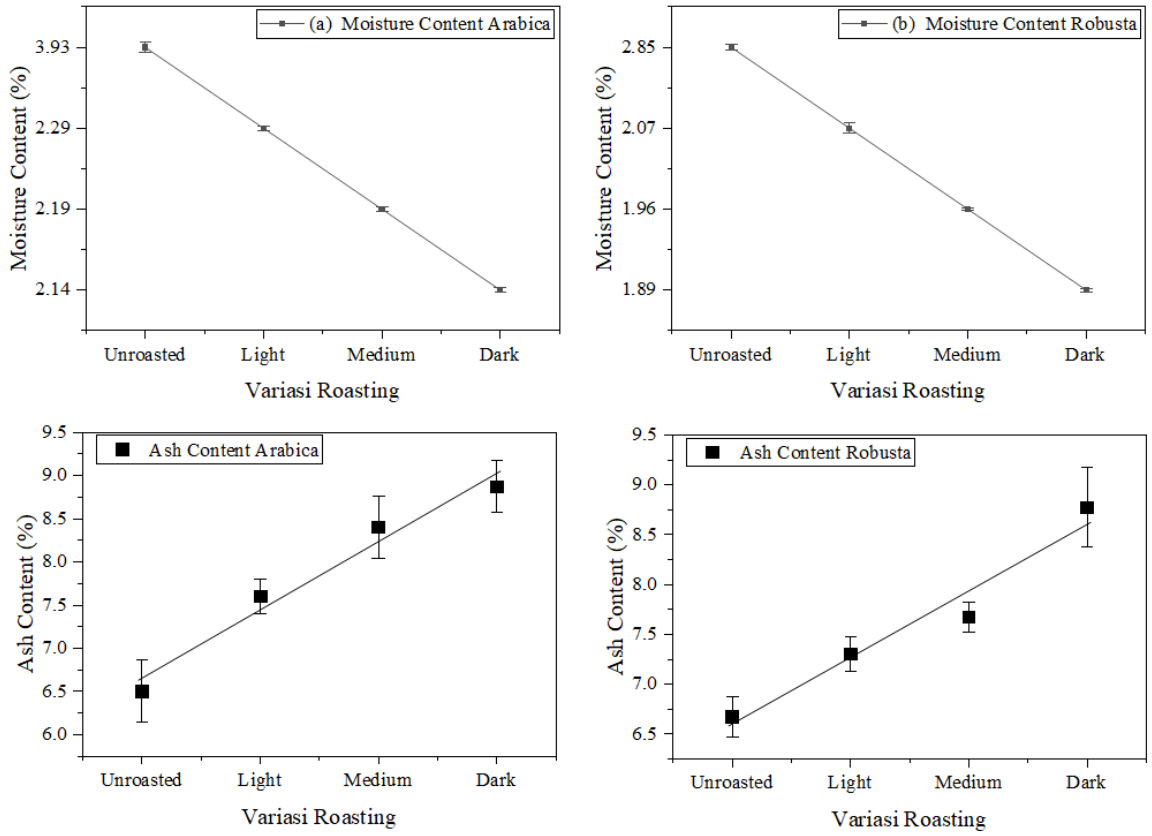


Figure 1. Moisture and Ash content of Arabica and Robusta under different roasting levels. (a) Moisture–Arabica, (b) Moisture–Robusta, (c) Ash–Arabica, (d) Ash–Robusta

Based on Figure 1, the highest ash content was recorded at 20 minutes of roasting, while the lowest was observed in green coffee beans. The ash content in green coffee beans was significantly lower compared to beans roasted for 10, 15, and 20 minutes. This indicates that roasting generally increases ash content, which is likely related to the reduction in moisture content and the resulting concentration of minerals within the coffee beans [23]. These results suggest an inverse relationship between moisture and ash content, where lower moisture levels correspond to higher ash values.

3.2.2. Determination of Acrylamide Content

The analysis of acrylamide levels in this study was conducted using the standard addition method, considering the complex coffee matrix that could potentially cause matrix effects on the analytical results. Therefore, the method was validated through testing for linearity, accuracy, precision, as well as LOD and LOQ values to ensure the reliability and accuracy of the measurements.

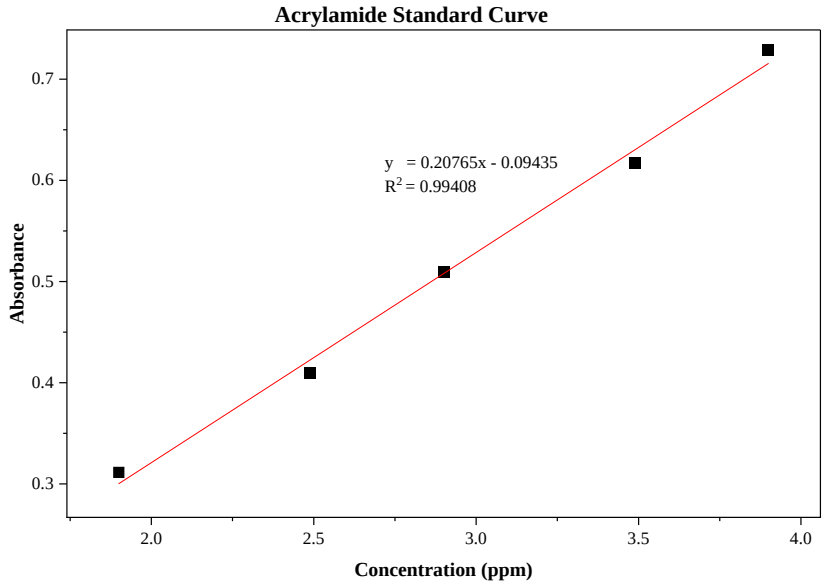


Figure 2. Calibration curve of acrylamide standard

Based on the calibration curve above, a linear correlation was obtained with the equation y = 0.20765x – 0.09435. The correlation between acrylamide concentration and instrument response was strong with a coefficient of determination (r^2) ≥ 0.998, indicating that the method is capable of providing a proportional response to changes in analyte concentration [19].

Table 2. Accuracy Percentage of Ethyl acetate fraction from Arabica and Robusta coffee roasted for 10 minutes

Sample	X (ppm)	Y	Measured Concentration (ppm)	Accuracy (%)	Mean (%)
Arabica 10	0	0.1511	1.1821		
		0.1544	1.1980		
		0.1567	1.2091		
	1.9	0.5373	3.0424	97.9109	96.7785
		0.5361	3.0366	96.7701	
		0.534	3.0265	95.6546	
	2.49	0.6577	3.6224	98.0028	96.9452
		0.6562	3.6151	97.0742	
		0.6517	3.5934	95.7587	
	2.9	0.6987	3.8198	90.9574	90.1047
		0.6943	3.7987	89.6784	
		0.6966	3.8097	89.6784	
	3.49	0.7844	4.2327	87.4091	87.1698
		0.7857	4.2389	87.1330	
		0.7868	4.2442	86.9674	
	3.9	0.8465	4.5318	85.8900	85.4248
		0.8457	4.5279	85.3836	
		0.8449	4.5241	85.0007	
Robusta 10	0	0.1845	1.3430		
		0.1826	1.3338		
		0.1833	1.3372		
	1.9	0.5287	3.0010	87.2629	86.8573
		0.5259	2.9875	87.0347	
		0.5236	2.9764	86.2742	

	0.6049	3.3680	81.3272	
2.49	0.6056	3.3714	81.8302	81.6947
	0.6068	3.3772	81.9269	
	0.7078	3.8637	86.9211	
2.9	0.7082	3.8656	87.3031	87.1093
	0.7077	3.8632	87.1038	
	0.7954	4.2856	84.3174	
3.49	0.7929	4.2736	84.2346	84.4968
	0.7987	4.3015	84.9385	
	0.8476	4.5371	81.9006	
3.9	0.8472	4.5352	82.0858	81.9623
	0.8464	4.5313	81.9006	

The accuracy of the method was evaluated by calculating the percent recovery using the standard addition method on sample matrices. Based on the results, the average % recovery of the ethyl acetate fraction of Arabica coffee ranged from 85.4248 to 96.9452%, while for Robusta coffee it ranged from 81.6947 to 87.1093%. Based on the criteria established by the Association of Analytical Chemistry (2015), the accuracy values obtained comply with the acceptable range of 80–110%, indicating that the UV-Vis spectrophotometric method used has good accuracy for the determination of acrylamide levels [24].

Table 3. Precision Percentage of Ethyl acetate fraction from Arabica and Robusta coffee roasted for 10 minutes

Sample	X (ppm)	Y	SD	Mean	Precision (%)
		0.6987			
Arabica 10	2.9	0.6943	0.0106	3.8094	0.2782
		0.6966			
		0.7078			
Robusta 10	2.9	0.7082	0.0013	0.0013	0.0329
		0.7077			

The precision test demonstrated the repeatability of the analytical results and was assessed using the relative standard deviation (RSD). In this study, the RSD values obtained for Arabica and Robusta coffee samples were both less than 2%, in accordance with the criterion stating that precision is considered acceptable when %RSD < 2% [25]. Therefore, the UV-Vis spectrophotometric method was proven to have good precision, meeting the established criteria for acrylamide analysis in coffee.

Table 4. LOD and LOQ values

Concentration (ppm)	Absorbance	yi	y-yi	$(y-yi)^2$
1.9	0.3113	0.3001	0.0111	1.2455×10^{-4}
2.49	0.4098	0.4226	0.0128	1.6435×10^{-4}
2.9	0.5091	0.5077	0.0013	1.8400×10^{-6}
3.49	0.6173	0.6302	0.0129	1.6692×10^{-4}
3.9	0.7290	0.7153	0.0136	1.8659×10^{-4}
Total				6.4427×10^{-4}
S (y/x)				1.2691×10^{-2}
LOD (ppm)				1.8340×10^{-1}
LOQ (ppm)				8.8343

The results showed that the LOD was 0.183 ppm and the LOQ was 8.83 ppm. The LOQ value being higher than the lowest standard concentration (4 ppm) indicates that the standard addition method used does not yet have sufficient sensitivity for quantification at low analyte levels, possibly

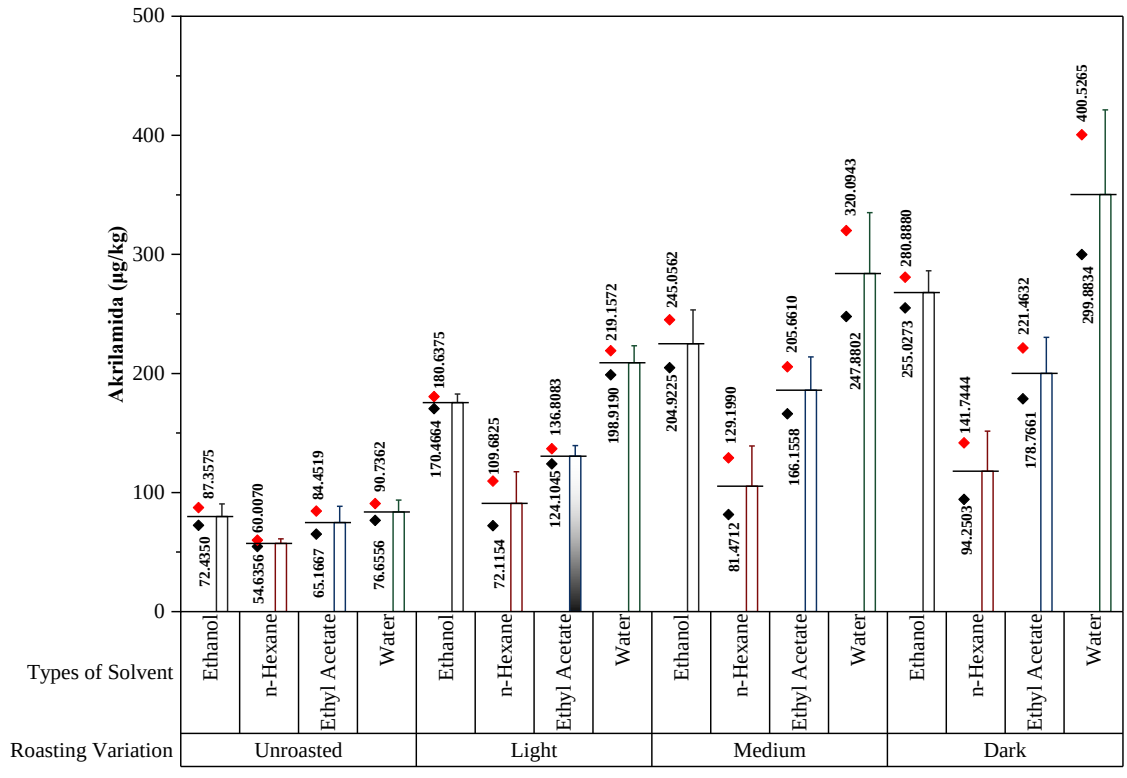
due to matrix effects. Nevertheless, the LOD being lower than the standard concentration indicates that the method is still capable of detecting the presence of acrylamide at low concentrations, although it is not yet optimal for quantification purposes.

Acrylamide is a compound that can form in carbohydrate-rich food materials, including coffee beans. The most important pathway for acrylamide formation is the decarboxylation and deamination of asparagine, which produces acrylamide. This process begins when asparagine reacts with a carbonyl compound to form a Schiff base, which then decomposes into acrylamide, particularly in the presence of α-hydroxy carbonyl compounds such as reducing sugars [26]. This pathway represents the initial stage of the Maillard reaction, which plays a crucial role in cooking processes and the development of food flavour [27].

In coffee, although the content of reducing sugars is relatively low, carbonyl compounds are still formed through sucrose degradation during heating and lipid oxidation. Sucrose breakdown produces glucose, fructose, and 5-hydroxymethylfurfural (HMF), with HMF being more effective than glucose in converting asparagine into acrylamide. In addition, the oxidation of linoleic acid, a major component of coffee oil, generates aldehydes and ketones that act as carbonyl precursors. Therefore, the availability of asparagine and carbonyl compounds, particularly HMF, is a key factor in acrylamide formation during the early stages of coffee heating or roasting [27].

The evaluation of acrylamide levels was conducted by selecting extracts and fractions from two types of coffee at different roasting levels. The results showed that the lowest acrylamide levels were found in coffee fractions extracted with n-hexane, ethyl acetate, ethanol, and water. This finding aligns with the polarity of acrylamide compounds. According to Souza et al. [28], acrylamide is highly polar and soluble in water, alcohol, acetone, and acetonitrile, slightly soluble in ethyl acetate and dichloromethane, but insoluble in hexane and other alkanes and alkenes. Additionally, acrylamide exhibits low yet significant volatility.

The following are the acrylamide content results in coffee beans across all roasting variations:



* Note: red shape= Robusta coffee; black shape= Arabica coffee

Figure 3. Comparison of Acrylamide Levels based on roasting variations, solvent and coffee types

Based on the graph above, Arabica and Robusta coffee bean samples showed an increase in acrylamide levels with longer roasting times across various solvents. The analysis revealed that the highest acrylamide levels were found in the water fraction at 20 minutes of roasting (dark roast), amounting to 299.8834 μg/kg in Arabica coffee and 400.5265 μg/kg in Robusta coffee. Conversely, the lowest acrylamide levels were observed in the n-hexane fraction at 10 minutes of roasting, measuring 72.1154 μg/kg in Arabica coffee and 109.6825 μg/kg in Robusta coffee. These results indicate that increased roasting intensity contributes to acrylamide formation in both coffee types.

Statistical analysis showed a p-value < 0.05 in the Kruskal-Wallis test, indicating a significant effect of roasting variations on acrylamide levels in both coffee types. However, further analysis revealed no significant difference between Arabica and Robusta coffees regarding acrylamide formation, as indicated by a p-value > 0.05 in the Mann-Whitney test. Dunn's post hoc test demonstrated significant differences between the treatment pairs dark–unroasted, dark–light, and medium–unroasted, indicating that increased roasting intensity has a notable impact on acrylamide formation. The statistical results confirm that roasting temperature and time are the main factors influencing acrylamide formation. Increased temperature and roasting duration can accelerate the Maillard reaction between amino acids and reducing sugars, contributing to acrylamide formation. Therefore, controlling roasting levels is a crucial aspect in efforts to minimize acrylamide content in coffee products.

Although the lowest acrylamide levels were obtained in the n-hexane fraction, solvent selection should not be based solely on acrylamide reduction, but also consider the functional benefits of the resulting extract. n-Hexane is nonpolar and thus less effective in extracting antioxidant compounds, which are generally polar. At 10 minutes of roasting, the ethyl acetate extract produced relatively lower acrylamide levels compared to longer roasting times, with values of 124.1045 μg/kg in Arabica coffee and 136.8083 μg/kg in Robusta coffee. The ethyl acetate fraction is also known to exhibit higher antioxidant activity than the n-hexane fraction and the water fraction [29].

In addition to affecting acrylamide formation, roasting time and temperature also influence the antioxidant content of coffee [30]. Changes in the antioxidant capacity of roasted coffee beans are associated with the degradation of chlorogenic acids, the formation of melanoidins, and the production of Maillard reaction products. During roasting, the Maillard reaction produces complex compounds with antioxidant activity through non-covalent interactions with phenolic compounds. One of the main products is melanoidin, whose levels can increase by up to 25%. In addition, caffeine also acts as an antioxidant compound in coffee beans. According to research by Fransiska et al. [31] roasting has a significant effect on the caffeine content of both green and roasted coffee beans. Higher degrees of roasting result in higher caffeine content. Caffeine is a compound that is stable under roasting temperatures, as it has a melting point of 238°C [32].

Based on the benchmark dose lower confidence limit ($BMDL_{10}$) approach, the reference values for acrylamide are 170 μg/kg body weight per day for carcinogenic effects and 430 μg/kg body weight per day for neurotoxic effects. The results of this study indicate that acrylamide levels in the ethyl acetate fraction with a roasting time of 10 minutes, ranging from 124.1045 μg/kg and 136.8083 μg/kg, lead to very low exposure levels, well below both $BMDL_{10}$ values [33]. Therefore, the use of ethyl acetate at 10 minutes of roasting is considered optimal, as it can minimize acrylamide formation without reducing antioxidant potential, making it suitable for nutraceutical capsule formulation.

3.3. Formulation and Evaluation of Nutraceutical Capsules

Nutraceuticals are products derived from food sources that provide health benefits and contain nutrients beneficial to the body [34]. They help improve health and well-being, modulate immunity, and prevent or treat various diseases and health conditions. Secondary metabolite compounds such as polyphenols, flavones, isoflavones, carotenoids, resveratrol, and coenzyme Q-10 play important roles in addressing a variety of health issues, including cancer, high cholesterol, hypertension, infections, diabetes, inflammation, osteoporosis, obesity, and in slowing the aging process. The majority of nutraceuticals exhibit biological activity as antioxidants, functioning through redox reactions to prevent chronic diseases and maintain oxidative balance in the body [35].

Granule evaluation in capsule preparations is necessary to assess granule quality and to determine their suitability for encapsulation. Granule evaluation includes organoleptic testing, moisture content determination, flow property testing (including moisture content, flow time, angle of repose, and compressibility), as well as content uniformity and disintegration time tests.

(a) (b)

Figure 4. a) Granules for the Nutraceutical Capsule Formulation of Arabica Coffee; (b) Granules for the Nutraceutical Capsule Formulation of Robusta Coffee.

The observational results indicate that Arabica coffee granules exhibit a brown colour with a strong caramel aroma and a granular form. Meanwhile, Robusta coffee granules show a lighter brown colour with a caramel aroma of lower intensity and a granular form similar to that of the Arabica sample.

Table 5. Evaluation of Arabica and Robusta Coffee Granules

Evaluation	Arabica				Robusta			
	R1	**R2**	**R3**	**Mean ±SD**	**R1**	**R2**	**R3**	**Mean ± SD**
MC (%)	1.63	1.82	1.43	1.63 ± 0.20	3.55	3.64	3.55	3.58 ± 0.05
FT (g/s)	0.75	0.73	0.79	0.76 ± 0.03	0.82	0.85	0.83	0.83 ± 0.01
AoR (º)	25.49	25.38	25.51	25.46 ± 0.07	25.48	25.38	25.51	25.46 ± 0.07
CI (%)	5.71	7.14	8.57	7.14 ± 1.43	10.00	13.33	10.00	11.11 ± 1.92

Notes: MC = Moisture Content, FT = Flowability Test, AoR = Angle of Repose, CI = Compressibility Index

Table 6. Physical Evaluation of Arabica and Robusta Coffee Nutraceutical Capsule

Parameter	Arabica capsule	Robusta capsule
Content uniformity (%)	6.34	8.34
Disintegration time (s)	4	3

The results of the study indicate that all granule evaluation parameters and physical evaluations of the capsules met the required testing standards. The produced granules exhibited appropriate organoleptic characteristics, moisture content within permissible limits, and good flow properties based on flow time, angle of repose, and compressibility tests. Similarly, the physical evaluation of the capsules, including content uniformity and disintegration time, showed results in accordance with established standards. Thus, the resulting capsule preparations meet quality requirements and are considered suitable for use as a nutraceutical dosage form.

4. CONCLUSION

This study demonstrates that a 10-minute roasting condition using the ethyl acetate fraction is optimal for both Arabica and Robusta coffee, as it results in lower acrylamide levels while preserving the extracted bioactive compounds. The formulated nutraceutical capsule product demonstrated acceptable physical quality in accordance with the required standards, indicating its suitability for further development as a dosage form. These findings support the selection of safe processing conditions without compromising the functional potential of coffee. The results provide a scientific basis for the formulation of low-acrylamide coffee products, particularly in nutraceutical capsule

form. Consequently, the developed products offer improved consumer safety while maintaining the health benefits of coffee-derived bioactive compounds.

Funding: The authors acknowledge the support of the Department of Pharmacy, Universitas Telogorejo Semarang, for providing research facilities.
Acknowledgments: The author expresses sincere gratitude to all lecturers of the Pharmacy Study Program, and especially to Mrs. apt. Firstca Aulia Rachma, M.Farm., and Mr. Dante Alighiri, M.Sc., as supervisors, for their guidance and support throughout this research. Thanks are also extended to all parties who have contributed to the completion of this study
Conflicts of interest: The authors declare no conflict of interest

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