Development of a Low-Cost Spectrophotometer for Protein Determination Using the Lowry Method

Lestha Aurel Salsabilla[1], Dadan Hermawan[1], Hartiwi Diastuti[1], Mekar Dwi Anggraeni[2], and Amin Fatoni[1*]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Jenderal Soedirman, Jl. Dr. Soeparno No. 61, Karangwangkal, Purwokerto 53123, Indonesia*

[2]*Department of Nursing, Faculty of Health Sciences, Universitas Jenderal Soedirman, Jl. Dr. Soeparno No. 61, Karangwangkal, Purwokerto 53123, Indonesia*

* ***Corresponding author:***

email: aminfatoni@unsoed.ac.id

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Abstract: *Affordable and accessible laboratory equipment is increasingly crucial, particularly in resource-limited settings. This research presents the development of a cost-effective spectrophotometer using an Arduino microcontroller and a TSL2591 light sensor to quantify protein concentration via the Lowry method. The device employs a 750 nm LED and a 3D-printed PLA case to house the components. Casein was used for calibration, yielding a linear detection range of 100–700 ppm with a high correlation coefficient (r = 0.9991). The limit of detection (LOD) and limit of quantification (LOQ) were 18.05 and 60.16 ppm, respectively. Precision was confirmed with a HORRAT value of 0.982. Accuracy was validated using the Wilcoxon Signed-Rank test, which showed no significant difference between the fabricated device and a commercial UV-vis spectrophotometer (p = 0.779). These findings support the reliability and reproducibility of the device for protein quantification. The project's open-source nature encourages further development and application in educational, clinical, and field settings. This innovation supports the development of accessible, low-cost biochemical analysis tools.*

Keywords: *Lowry method; protein analysis; spectrophotometer alternative*

■ INTRODUCTION

The continuous development of chemistry instrumentation plays a pivotal role in advancing scientific research and improving the quality of life [1]. Innovations in this field have led to more precise and cost-effective solutions, enabling researchers and practitioners to achieve accurate results while minimizing expenses [2]. Historically, sophisticated instruments like spectrophotometers were primarily available in well-funded laboratories, limiting access in resource-limited settings. However, advancements in electronics, sensors, and microcontroller technology have driven the development of compact and affordable devices that maintain reliable performance [3]. These breakthroughs enhance scientific capabilities and democratize access to essential tools, allowing a wider range of institutions, from universities to small-scale laboratories, to participate in cutting-edge research and diagnostics.

As the global demand for accessible and affordable scientific tools continues to grow, particularly in developing countries, the need for innovative, low-cost alternatives becomes increasingly essential [4-5]. This initiative is underpinned by the recognition that scientific progress should be accessible to all, regardless of financial means. In resource-limited settings, affordable instrumentation can significantly impact fields such as healthcare, agriculture, and environmental monitoring, where accurate and timely analysis is crucial but often inaccessible. By reducing the financial burden of acquiring laboratory equipment, low-cost innovations can empower local researchers and practitioners to address pressing global challenges, improve healthcare outcomes, and foster sustainable development. Furthermore, these accessible solutions contribute to the global scientific community by enabling collaborative

research and knowledge exchange between regions with varying resource levels. This inclusivity helps bridge gaps in scientific progress and fosters a more equitable distribution of scientific advancements.

Spectrophotometers are among the most widely used instruments in chemistry to determine various analytes [6-9]. These devices measure the intensity of light absorbed or transmitted by a sample at specific wavelengths, providing critical data for quantitative analysis in numerous applications. Whether analyzing the concentration of a substance in solution or monitoring the purity of a chemical reaction, spectrophotometers offer a precise, reliable method to detect and quantify materials. Their versatility makes them indispensable in fundamental research and industrial processes, allowing scientists to explore chemical properties with accuracy and reproducibility. In addition, their adaptability to various wavelengths and configurations ensures that they remain a cornerstone of analytical chemistry, addressing the evolving needs of modern science.

From environmental monitoring to clinical diagnostics [10-11], spectrophotometers are crucial in assessing everything from water quality to blood analytes, contributing to public health, environmental sustainability, and process optimization. In environmental science, they enable the detection of contaminants such as heavy metals, nitrates, and organic pollutants in water and soil. In healthcare, they are essential for diagnosing diseases by measuring specific biomarkers, facilitating early detection and treatment. Additionally, spectrophotometers are widely used in the pharmaceutical industry for quality control, ensuring that drugs meet regulatory standards and are safe for consumption [12]. These applications underscore the instrument's central role in addressing challenges that impact societal well-being, emphasizing its significance beyond the laboratory.

Moreover, their applications extend to food and beverage industries, where they are used to analyze the composition of products [13], and in agriculture, where they help monitor nutrient levels in soil or plant tissues [14]. The broad utility of spectrophotometers underscores their significance in modern science and industry, providing a foundation for countless chemical, biological, and physical investigations [15]. Their ongoing evolution, driven by the need for more affordable and portable versions, continues to expand their reach, making these essential tools more accessible to researchers and professionals around the globe. The integration of compact spectrophotometers into fieldwork further highlights their transformative potential, as portable devices enable on-site analysis, reducing the need for extensive sample transportation and enhancing data reliability in remote or challenging environments [16].

Protein assays, such as the Lowry method, are prime examples of biochemistry applications that rely heavily on spectrophotometers. The Lowry method is a well-established and widely used technique for quantifying protein concentration in a sample, based on the colorimetric detection of protein-copper complexes that form under specific conditions [17]. During the assay, proteins in the sample react with copper ions in an alkaline solution, forming a complex that subsequently reacts with the Folin-Ciocalteu reagent, producing a blue color. The intensity of the blue color, which is directly proportional to the protein concentration, is measured by a spectrophotometer, allowing for precise quantification.

This method is essential in numerous biological, biochemical, and medical research areas, where accurate protein measurement is crucial for understanding cellular functions, enzymatic activities, and disease mechanisms [18-19]. For instance, in molecular biology and biochemistry laboratories, protein assays like the Lowry method are routinely used to study enzyme kinetics, protein expression levels, and post-translational modifications. In medical research, protein concentrations are critical for diagnosing diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions, where protein biomarkers provide key insights into disease progression and therapeutic responses. The widespread application of these assays emphasizes the necessity of making such methods affordable and accessible to promote advancements in global health research.

In recent years, researchers have explored various low-cost alternatives to traditional spectrophotometers.

These alternatives include using digital images captured by cameras [20-22] or scanners [23-24], as well as employing color sensors and light sensors [25]. Such innovations are particularly valuable in low-income regions, where access to expensive laboratory equipment is limited [26-27]. These cost-effective solutions can facilitate scientific research and education, bridging the gap in resource availability and enabling broader participation in scientific endeavors. By leveraging locally available materials and accessible technologies, these innovations contribute to capacity building and foster a culture of self-reliance in scientific development.

The proposed spectrophotometer offers multiple advantages: it is low-cost, easy to construct using open-source components, and capable of delivering reliable analytical performance comparable to commercial devices. It uses minimal power, features a compact and portable design, and can be reproduced easily for educational or field-based use, making it highly suitable for underfunded laboratories and remote areas. This study aims to develop an affordable spectrophotometer using the TSL2591 light sensor, a 750 nm LED (selected to align with the peak absorbance range of the Lowry method, typically between 660–750 nm), and a microcontroller for protein determination via the Lowry method. The 750 nm wavelength was chosen due to component availability, reduced background interference, and improved sensitivity in detecting absorbance changes—particularly in turbid or colored samples. By leveraging these inexpensive components, we propose a practical and accessible solution for protein analysis that can be implemented in various settings. This innovation aligns with several Sustainable Development Goals (SDGs), particularly SDG 3 (good health and well-being), SDG 4 (quality education), and SDG 9 (industry, innovation, and infrastructure) [28]. It enables cost-effective protein analysis to support healthcare systems in resource-limited settings, improving diagnostic capabilities and research capacity. Moreover, the integration of affordable technologies into educational curricula enhances the quality of science education, empowering students and researchers to develop local solutions to global challenges.

■ EXPERIMENTAL SECTION

Materials

Materials used for protein assay were obtained from Sigma-Aldrich including casein from bovine milk, copper(II) sulfate, sodium tartrate, sodium carbonate, and Folin-Ciocalteu reagent, as well as commercial milk sample. The materials used for cuvette holder and spectrophotometer cover fabrication were polylactic acid (PLA) filament (CR-PLA, Shenzhen Creality 3D Technology Co, Ltd.) using a 3D Printer (Ender 3 Pro, Shenzhen Creality 3D Technology Co, Ltd.).

Instrumentation

The low-cost spectrophotometer was fabricated using the TSL2591 Light Sensor (Adafruit) 750 nm LED (Vishay Semiconductors, TSAL6100), microcontroller (Arduino Uno R3), assorted jumper wires and connectors. The protein measurement comparison was performed using a commercial spectrophotometer (Shimadzu 1600 UV-vis Spectrophotometer, Japan).

Procedure

Cost-effective spectrophotometer fabrication

The design and fabrication process of the low-cost spectrophotometer began with careful planning of the cuvette holder and the main housing using 3D modeling software, specifically Tinkercad. This software was chosen for its freeware, simple and intuitive interface, allowing for precise design of the components, ensuring proper alignment and functionality in the final assembly. The cuvette holder was designed to securely position the sample in the path of the light beam. At the same time, the housing was crafted to accommodate all internal components, including the light sensor, LED, and microcontroller, with sufficient space for wiring and ventilation.

The designs were then printed using a 3D printer, utilizing PLA filament. PLA was selected not only for its ease of printing and flexibility in handling but also for its environmentally friendly properties, as it is biodegradable and derived from renewable resources. The 3D-printed parts were produced with high precision to ensure a fit between the cuvette holder, the sensor,

and other critical components. Post-printing, the parts were carefully cleaned and checked.

The assembly involved integrating the TSL2591 light sensor, which can detect light across a wide range of wavelengths with high sensitivity, into the 3D-printed housing. A 750 nm LED was chosen as the light source for its specific wavelength to match the absorption maximum of the protein-copper complexes in the Lowry assay. An Arduino UNO R3 microcontroller served as the device's brains, controlling the LED and reading data from the light sensor. The components were positioned precisely to ensure that the light passed directly through the cuvette and was detected by the sensor without interference or scattering.

Wiring was performed and each connection tested for continuity and strength, as loose or faulty wiring could compromise the accuracy of the measurements. The code for controlling the LED and reading the data from the TSL2591 sensor was written in the Arduino Integrated Development Environment (IDE), using libraries specific to the sensor. This code enabled the microcontroller to regulate the LED's power output and collect absorbance data from the sensor in real time. After the code was uploaded to the microcontroller, initial tests were conducted to validate the functionality of the assembled spectrophotometer. These tests included checking the stability of the LED output, the responsiveness of the light sensor to changes in absorbance, and the overall accuracy of the measurements. Minor adjustments were made to optimize the alignment and improve signal quality.

Standard casein measurement

Standard solutions of casein protein were prepared with concentrations of 100, 200, 300, 400, 500, 600, 700, and 800 ppm to create a calibration curve for the protein assay. Each standard solution was precisely measured into 1 mL aliquots and transferred into clean test tubes for analysis. To each 1 mL of casein solution, 5 mL of Lowry reagent C. This reagent was freshly prepared by mixing 2 mL of reagent B (0.5 g of $CuSO_4{\cdot}5H_2O$ dissolved and diluted to 100 mL with 1% sodium potassium tartrate) with 100 mL of reagent A (2 g of Na_2CO_3 dissolved and diluted to 100 mL with 0.1 M NaOH). The mixture was vortexed and allowed to stand at room temperature for 10 min, allowing the protein-copper complexes to form fully. Subsequently, 0.5 mL of reagent D was added rapidly and mixed thoroughly to ensure complete mixing of the solution, promoting uniform reaction conditions throughout the sample. Reagent D was prepared by diluting Folin–Ciocalteu reagent in a 1:1 ratio with distilled water (5 mL of reagent diluted to 10 mL with water). The reaction mixture was then incubated at room temperature for 30 to complete the color development process. During this time, the blue complex stabilized, allowing for accurate absorbance readings. Absorbance was measured at 750 nm using both the fabricated spectrophotometer and a commercial UV-vis spectrophotometer.

To ensure the reliability and validity of the results, the data obtained from both the fabricated and commercial spectrophotometers were analyzed statistically. The Wilcoxon Signed-Rank, non-parametric statistical test was employed to compare the paired absorbance measurements from the two instruments. This test was chosen to determine whether there were any statistically significant differences between the two sets of data, offering insights into the accuracy and precision of the fabricated spectrophotometer compared to the commercial instrument.

Determination of linearity, limit of detection, and limit of quantification

The linearity of the fabricated spectrophotometer was evaluated by analyzing the correlation coefficient (r) of the calibration curve obtained from the standard protein solutions. A high R^2 value indicates good linearity. The limit of detection (LOD) and limit of quantification (LOQ) were calculated using standard statistical methods. The LOD was determined as the concentration corresponding to three times the standard deviation of the regression line intercept divided by the slope (Eq. (1)). In comparison, the LOQ was determined as ten times the standard deviation of the regression line intercept divided by the slope (Eq. (2));

$$\mathrm{LOD} = \frac{3 \times \mathrm{SD}}{\mathrm{b}} \quad (1)$$

$$\mathrm{LOQ} = \frac{10 \times \mathrm{SD}}{\mathrm{b}} \quad (2)$$

where SD was the standard error of intercept and b was the slope of the regression line.

Determination of the precision of the Lowry method

A standard solution of casein protein, with a concentration of 300 ppm, was aliquoted into six different test tubes. Each sample was then reacted with the Lowry reagent according to the protein measurement in the previous method. The absorbance of the resulting solutions was measured using the fabricated spectrophotometer. The analysis included calculating the standard deviation (SD) and the percentage of relative standard deviation (%RSD), also known as the coefficient of variation (CV), using Eq. (3) and (4).

$$\%\frac{\text{RSD}}{\text{KV}} = \frac{\text{SD}}{\tilde{\text{x}}} \times 100\% \quad (3)$$

$$\text{CV theoretic} = 2^{(1-0.5\log \text{C})} \times 0.67 \quad (4)$$

$$\text{HORRAT} = \frac{\text{CV experiment}}{\text{CV theoretic}} \quad (5)$$

Spectrophotometer precision determination

A standard solution of casein protein with a concentration of 300 ppm was prepared and placed into a test tube for analysis. Absorbance measurements were taken using six simple spectrophotometers, each designed to offer a low-cost alternative for protein quantification. These spectrophotometers were tested under the same experimental conditions to ensure consistency in data collection. The absorbance values from each device were recorded and analyzed to evaluate their precision and reliability. By comparing the performance of multiple spectrophotometers, it was possible to assess the reproducibility of the results, a critical factor in determining the viability of these instruments for routine analytical use.

Following data collection, statistical analysis was performed to quantify the variability across the six devices. The SD of the absorbance readings was calculated, providing insight into the spread of the data and the precision of each spectrophotometer. Additionally, the %RSD, also known as CV, was determined to assess the relative variability in the measurements. To further validate the reliability of the results, the HORRAT value was calculated using the specified equation (Eq. (5)). The combination of these statistical tools allowed for a comprehensive evaluation of the spectrophotometers' performance, ensuring their capability to deliver consistent and accurate results in protein quantification applications.

Determination of accuracy

A protein sample solution was prepared, and 5 mL was aliquoted into six 10 mL measuring flasks. As much as 1.0 mL of a standard casein solution, derived from a 1000 ppm casein stock solution was added to each flask. The solutions were then diluted to the mark with distilled water and thoroughly mixed. Subsequently, 1 mL of each solution was taken and reacted with the Lowry reagent. The protein content was measured using a simple spectrophotometer. Protein levels were calculated based on the regression equation from the linearity curve. Accuracy was calculated using Eq. (6) (C_p: total concentration, C_a: sample concentration, and $C_{a'}$: analytes added concentration).

$$\%\,\text{Recovery} = \frac{C_p - C_a}{C_{a'}} \times 100\% \quad (6)$$

Determination of protein in real sample

A goat's milk protein sample solution was prepared and carefully placed into a clean test tube for analysis. To initiate the Lowry protein assay, five milliliters of Lowry reagent, a mixture of copper sulfate and tartrate in an alkaline solution, were added to the protein sample. The addition of this reagent enables the formation of protein-copper complexes, which are central to the colorimetric reaction. The test tube was gently shaken to ensure the solution was thoroughly mixed, promoting uniform reaction conditions. The mixture was then left to incubate at room temperature for 10 min to allow the protein-copper complex formation to proceed efficiently.

Following this incubation, 0.5 mL of Folin-Ciocalteu reagent, a phosphomolybdic-phosphotungstic acid solution, was quickly added to the mixture. The rapid addition of the Folin reagent is crucial for reducing copper ions and producing a blue-colored complex. After thoroughly mixing the solution to ensure even distribution of the reagents, the test tube was left to stand at room temperature for an additional 30 min. This

incubation period allows the color development to stabilize, with the intensity of the blue color corresponding to the protein concentration in the sample.

The absorbance of the sample was then measured at a wavelength of 750 nm, where the blue complex exhibits maximum absorption. The absorbance readings were taken using two different instruments: a fabricated low-cost spectrophotometer and a commercially available, high-precision spectrophotometer. This dual measurement approach provided a comparative analysis of the accuracy and reliability of the fabricated device against the standard commercial instrument. By assessing the absorbance at the same wavelength, the performance of the fabricated spectrophotometer could be validated, offering insights into its potential for cost-effective protein quantification in various settings.

■ RESULTS AND DISCUSSION

Cost-Effective Spectrophotometer Fabrication

A simple spectrophotometer was designed using a red LED light source with a wavelength of 750 nm. The light from the LED was detected by a TSL2591 light sensor, which was connected to an Arduino UNO microcontroller. The TSL2591 light sensor served as a light-to-digital converter, translating the intensity of light into digital signals that could be processed by the microcontroller. The solution holder was fabricated using PLA filament material with a 3D printer and was designed to be tightly closed to prevent external light from interfering with the detection process by the light sensor. This design ensured that measurements were accurate and reliable by eliminating potential sources of error due to ambient light.

The choice of a red LED with a 750 nm wavelength was deliberate, as it is suitable for many biochemical assays, including protein analysis using the Lowry method. The wavelength falls within the optimal range for detecting the colorimetric changes associated with protein concentration measurements. This wavelength ensures sufficient sensitivity and minimizes interference from other potential chromophores that might absorb light outside this range. Additionally, using a single-wavelength source simplifies the hardware design and reduces costs compared to more complex spectrophotometers employing multiple wavelengths.

The device's compact design and affordability make it an excellent tool for educational purposes and preliminary research applications. Its portability further enhances its utility, enabling users to perform protein analysis in various settings, including fieldwork or laboratories with limited resources. The device was constructed as shown in Fig. 1, demonstrating a practical implementation of integrating modern electronics with traditional biochemical analysis techniques. The use of a 3D printer for fabrication also highlights the adaptability and scalability of the design, as researchers can customize the holder for different sample types or experimental conditions. This feature underlines the device's versatility and potential for further modification to suit specific analytical needs.

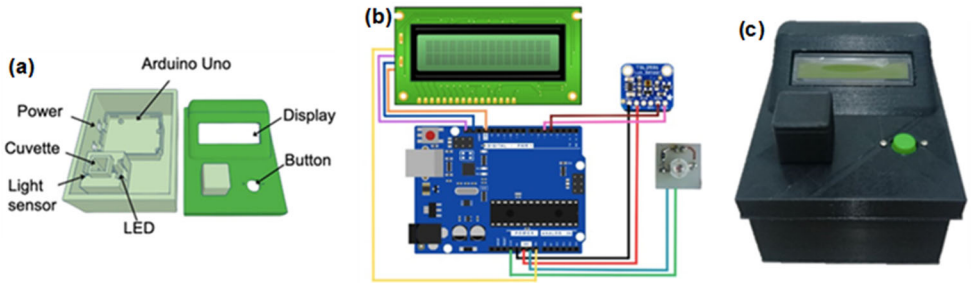

Fig 1. Comprehensive view of the cost-effective spectrophotometer designed for affordable protein analysis using the Lowry method. (a) 3D rendering showcasing the design details and assembly components; (b) schematic diagram highlighting key internal components and their interconnections; (c) photograph of the fully printed and assembled spectrophotometer, demonstrating its practical implementation

Standard Protein Measurement and Performance Comparison

The Lowry method test is based on the reduction of Cu^{2+} to Cu^{+} by proteins. The Cu^{+} ions then react with the Folin-Ciocalteu reagent to form a blue color due to the production of hetero-polymolybdenum blue, which can be detected at wavelengths between 500 and 750 nm [17]. This colorimetric change allows for the quantification of protein concentration based on the intensity of the blue color formed. To validate this method, standard solutions of casein protein with concentrations of 100, 200, 300, 400, 500, 600, 700, and 800 ppm were prepared and reacted using the Lowry method. The absorbance of these solutions was measured using a UV-vis spectrophotometer, which provides precise and accurate readings of absorbance. Additionally, the light intensity of the solutions was measured using a simple spectrophotometer with a wavelength of 750 nm. This comparison aimed to evaluate the performance and reliability of the fabricated spectrophotometer against the standard UV-vis spectrophotometer. The results of the light intensity measurements using the simple spectrophotometer and the absorbance measurements using the UV-vis spectrophotometer were plotted to create a calibration curve, relating the light intensity from the simple spectrophotometer to the absorbance from the UV-vis spectrophotometer. Fig. 2 shows a calibration curve for the simple spectrophotometer.

From the plotted data, an exponential regression model was found to best fit the experimental results, with the resulting equation: $y = 346.8e^{-2} \times 418x$. This model produced a coefficient of determination (R^2) of 0.9998 and a r of 0.9999, indicating an excellent correlation between the light intensity measurements and the reference absorbance values. Compared to a linear regression, the exponential model significantly improved the goodness-of-fit, making it more appropriate for the system's optical response curve.

In addition to its better statistical performance, the exponential function was selected because it reflects the natural decay behavior of transmitted light intensity in relation to increasing absorbance. Furthermore, this regression model could be easily integrated into the Arduino IDE without computational burden, allowing real-time conversion of light intensity values into absorbance. This automated calculation reduces potential human errors in data processing and ensures consistency in results. Table 1 summarizes the absorbance measurements obtained using both the UV-vis spectrophotometer and the simple spectrophotometer. The comparison highlights the fabricated spectrophotometer's capability to accurately measure protein concentrations across a wide range, further demonstrating its potential as a cost-effective alternative.

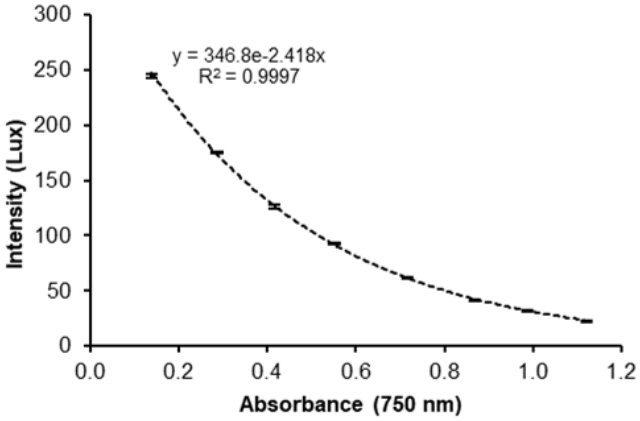

Fig 2. Comparison of light intensity measurements from the fabricated spectrophotometer to absorbance readings from the UV-vis spectrophotometer for measuring 100–800 ppm protein. The y-axis represents the light intensity measured by the simple spectrophotometer, while the x-axis represents the absorbance values obtained from the standard UV-vis spectrophotometer

The measurement results of the casein standard solution obtained from both the UV-vis spectrophotometer and the fabricated simple spectrophotometer were subjected to statistical analysis using the Wilcoxon Signed Rank test. The test yielded a Z value of −0.28; the significance level obtained was 0.779. The Wilcoxon Signed Rank test, a non-parametric test, is used to compare paired samples when the distribution of differences between paired observations is not normally distributed. In this case, a significance level greater than 0.05 indicates no significant difference between the absorbance measurements obtained from

Table 1. Absorbance measurements of standard protein solutions: comparison between standard UV-vis spectrophotometer readings and calculated absorbance values from the fabricated simple spectrophotometer

Concentration (ppm)	Absorbance		Diff.	Wilcoxon Signed Rank test			
	Commercial UV-vis spectrophotometer	Fabricated spectrophotometer		Abs. Diff.	Rank	Pos. Rank	Neg. Rank
100	100.000	102.290	−2.290	2.290	4		4
200	205.710	203.000	2.710	2.710	5	5	
300	298.570	302.290	−3.720	3.720	7		7
400	395.570	389.430	6.140	6.140	8	8	
500	512.860	514.430	−1.570	1.570	3		3
600	621.430	624.430	−3.000	3.000	6		6
700	705.710	705.140	0.570	0.570	1	1	
800	802.290	801.430	0.860	0.860	2	2	
			Smaller sum = 16				
			Critical point (0.05) = 3				

the UV-vis spectrophotometer and those calculated using the simple spectrophotometer.

These results suggest that the simple spectrophotometer, despite its basic design, provides reliable and comparable measurements of protein levels compared to the more advanced UV-vis spectrophotometer. The low-cost design, ease of use, and high accuracy make this spectrophotometer invaluable for resource-limited settings, education, and preliminary research. Its potential for further customization also paves the way for expanding its applications to other biochemical assays, enhancing its versatility as an analytical tool.

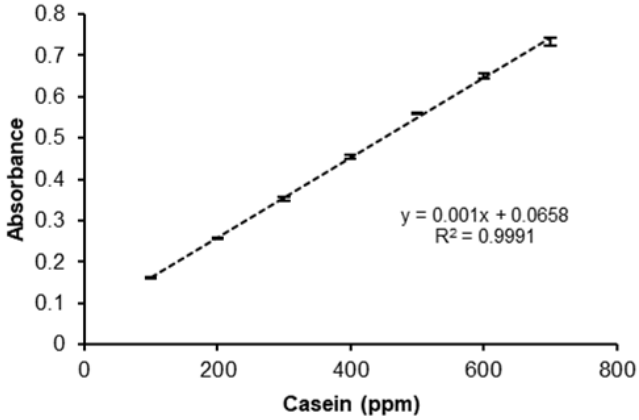

Fig 3. The calibration curve of standard casein solutions was measured using the fabricated spectrophotometer

Linearity, LOD and LOQ Determination

Linearity, LOD, and LOQ are fundamental parameters in analytical method validation. They ensure the method can produce accurate and consistent results over a specified range of analyte concentrations. These parameters are critical in analytical chemistry, clinical diagnostics, and environmental science, where precision and accuracy are paramount for reliable data interpretation [4]. In this study, the linearity of the fabricated spectrophotometer was assessed using casein standard solutions with concentrations ranging from 100 to 700 ppm. Measurements were conducted, and the resulting calibration curve (Fig. 3) adhered to the Lambert-Beer law, confirming a direct relationship between absorbance and concentration.

The regression analysis of the calibration data yielded a linear regression equation y = 0.001x + 0., with a R^2 of 0.9991 and a r of 0.9995. These values demonstrate an excellent linear relationship and high precision in the spectrophotometer's readings. The calculated LOD was 56.751 ppm, while the LOQ was 189.17 ppm, indicating the method's sensitivity and capability to accurately detect and quantify low concentrations of casein. These relatively high values reflect the limitations of the device in detecting very low concentrations of protein, particularly when compared to conventional UV-vis spectrophotometers, which typically achieve LODs below 10 ppm. This limitation is likely influenced by the lower sensitivity of the light sensor used and the simplified optical path design.

Furthermore, the dynamic range of the spectrophotometer was established between the LOQ and the upper limit, corresponding to a concentration of 3011.78 ppm. The upper limit was determined as the concentration at which light absorbance reached saturation. This broad range enhances the versatility of the method, allowing for its application in scenarios requiring the quantification of proteins across diverse concentration levels. The ability to maintain high accuracy over this range highlights the robustness of the fabricated spectrophotometer in analytical applications.

Precision Determination

Precision evaluation is vital for assessing the reproducibility of analytical methods and ensuring consistent results across multiple measurements. This study evaluated the precision of the Lowry method and the fabricated spectrophotometer, employing rigorous statistical measures to validate their reliability. Precision was determined by calculating the %RSD for repeated measurements, a key indicator of variability within a data set.

For the Lowry method, six independently prepared casein standard solutions (300 ppm) were analyzed under identical conditions (Table 2). The resulting %RSD was 1.114%, a remarkably low value that reflects minimal variability in the measurements. This demonstrates the method's high reproducibility and robustness, which are critical qualities for any analytical technique used in protein quantification. Additionally, the HORRAT (Horwitz Ratio) value was calculated to be 0.831, which falls well within the acceptable range of 0.3 to 2.0 for reproducibility in chemical analysis. This further validates the reliability of the Lowry method, confirming its compliance with established performance criteria and its suitability for both research and industrial applications.

For the fabricated spectrophotometer, precision was assessed by measuring the absorbance of a single casein standard solution (300 ppm) across six different devices. This approach was specifically designed to evaluate inter-device variability, a critical consideration for practical deployment in diverse settings where multiple device units might be used. The %RSD for these measurements was 1.098%, closely matching the precision observed with the Lowry method. The low %RSD value indicates that the fabricated spectrophotometer can produce highly consistent results, even when measurements are conducted using different units. Similarly, the HORRAT value for the fabricated device was 0.82, further emphasizing its reliability and alignment with standard performance benchmarks [29].

Table 2. Replicate measurement results and precision evaluation of casein protein absorbance using the fabricated spectrophotometer (n = 6)

Replicate	Absorbance	$(x-\bar{x})$	$(x-\bar{x})^2$
1	0.366	-0.002	0.000004
2	0.366	-0.002	0.000004
3	0.369	0.001	0.000001
4	0.375	0.007	0.000049
5	0.363	-0.005	0.000025
6	0.369	0.001	0.000001
	$\bar{x} = 0.368$		$\sum = 0.000084$
		SD	0.004099
		RSD/CV	1.114%
		HORRAT	0.831

The precision demonstrated by the Lowry method and the fabricated spectrophotometer is noteworthy. The low %RSD values highlight the minimal experimental error inherent in these methods, ensuring that the data obtained is consistent and reproducible. Furthermore, HORRAT values close to 1.0 provide additional assurance that the methods meet international standards for precision in analytical chemistry. These metrics are particularly important when the methods are used in sensitive applications, such as quality control in food science or detailed biochemical analyses in research settings.

Importantly, the high precision observed for the fabricated spectrophotometer underscores its potential as a reliable, cost-effective alternative to more conventional methods like the Lowry assay. Its ability to maintain consistency across different devices is especially valuable for applications in resource-limited settings, where affordability and ease of deployment are essential. This reliability also enhances the device's versatility, making it suitable for various applications, from educational laboratories to industrial quality control processes.

The precision evaluation confirmed the reliability of both the Lowry method and the fabricated spectrophotometer. The comparable %RSD values and HORRAT ratios for the two methods highlight their capability to deliver consistent and reproducible results. These findings not only validate the fabricated spectrophotometer as a precise analytical tool but also establish its practicality for widespread use in protein quantification, bridging the gap between traditional laboratory methods and modern, cost-effective innovations.

Accuracy Determination

Accuracy assessment in this study was conducted using the standard addition method, a widely recognized approach for evaluating the reliability of analytical measurements. In this method, a known amount of casein standard solution (100 ppm) was added to six different samples. The primary objective of this approach is to assess how effectively the analytical method recovers the added standard, providing a clear indication of the method's accuracy. By comparing the measured concentrations before and after the addition of the standard, any potential systematic errors or biases in the method can be identified and quantified.

The results of the accuracy assessment are presented in Table 3, showing an average recovery value of 102%. This recovery rate indicates that the method achieves a close approximation to the true concentration of casein in the samples, demonstrating good accuracy [30]. Recovery values close to 100% are generally considered optimal, as they reflect minimal systematic errors in the analytical process. The range of recovery percentages in this study (98 to 106%) further underscores the consistency and reliability of the method. Such values suggest that the technique can accurately quantify casein content without significant deviation, even in a complex sample matrix.

Accurate determination of protein concentrations is critical across various scientific disciplines, including biochemical analysis, food science, and nutritional research. The obtained recovery value of 102% highlights the method's robustness and reliability in quantifying casein content with high precision. This level of accuracy ensures the validity and reproducibility of experimental results, making the method suitable for diverse applications. For instance, precise protein quantification in food science is essential for quality control, nutritional labeling, and formulation of dairy and other protein-based products. The ability to confidently measure protein levels enhances product consistency, supports regulatory compliance, and ensures consumer trust.

Table 3. Accuracy assessment using the standard addition method for casein determination

Standard added (ppm)	Concentration (ppm)			%Recovery
	Sample	Calculated	Found	
100	279.1	379.1	385.1	106.0
100	279.1	379.1	382.1	103.0
100	279.1	379.1	378.1	99.0
100	279.1	379.1	377.1	98.0
100	279.1	379.1	381.1	102.0
100	279.1	379.1	383.1	104.0
		Average		102.0

The effectiveness of the standard addition method in this study is particularly noteworthy. This approach validates the analytical method's accuracy and helps identify potential interferences that may arise from the sample matrix. By spiking samples with a known standard, researchers can ensure that the method accurately accounts for any matrix effects, which is crucial when dealing with complex biological or food samples. The recovery values obtained in this study suggest that matrix effects were minimal, further confirming the method's reliability.

Additionally, the recovery results provide critical evidence for the method's applicability in both research and industrial settings. For researchers, the ability to obtain accurate and reproducible data is foundational for advancing scientific understanding and developing new technologies. For industrial applications, such as routine quality control in food manufacturing, the method's demonstrated accuracy ensures that products meet strict nutritional and safety standards.

In conclusion, the accuracy assessment using the standard addition method demonstrates the analytical method's capability to recover added casein standards with minimal error. With an average recovery of 102%, the method has proven to be a reliable tool for protein

quantification, reinforcing its suitability for a wide range of scientific and industrial applications. These findings emphasize the importance of thorough accuracy validation in analytical methods, ensuring confidence in the results and their broader implications for research, quality assurance, and regulatory compliance.

Determination of Protein in Real Sample

Protein content in real samples of goat milk was measured using a fabricated low-cost spectrophotometer and compared with a standard UV-vis spectrophotometer. The determination of protein content in goat milk is a crucial parameter for evaluating its nutritional value, quality, and overall suitability for human consumption. Protein levels in milk are often indicative of its health benefits, serving as a vital component in assessing the nutritional properties of dairy products and their potential applications in diverse industries such as food, pharmaceuticals, and functional nutrition [31]. High-protein milk is often associated with enhanced dietary benefits, making accurate and reliable protein quantification essential for maintaining product standards and meeting consumer demands.

In this study, a fabricated low-cost spectrophotometer and a standard UV-vis spectrophotometer were employed to quantify protein levels in goat milk samples. The samples were prepared by dissolving 1 g of powdered goat milk in 100 mL of distilled water, representing a standard dilution commonly used in dairy analysis. The fabricated spectrophotometer demonstrated the capability to deliver precise measurements, with an average protein content of 415.1 ppm (Table 4), showcasing its potential to analyze complex biological matrices like goat milk effectively. Although reconstituted milk reduces the complexity of the sample matrix compared to fresh or whole milk, potential matrix interferences may still arise. Components such as residual fat, minerals, or additives in milk powder could influence the Lowry reaction or light transmission during absorbance measurement. These matrix effects may introduce minor variability, though no pretreatment beyond dilution was applied in this study.

Table 4. Protein analysis in goat milk using a low-cost and commercial spectrophotometer

Samples	Concentration (ppm)	
	Los-cost spectrophotometer	Commercial spectrophotometer UV-vis
1	413.1	414.29
2	416.1	416.43
3	416.1	417.14
4	413.1	413.57
5	418.1	417.86
6	414.1	414.29
Average	415.1	415.60

Despite these potential interferences, the fabricated device produced results in close agreement with the commercial UV-vis spectrophotometer, indicating that the matrix effects in the reconstituted samples were minimal or manageable under the conditions tested. These results underscore the practicality and versatility of the fabricated device in real-world applications, particularly in situations where high-cost laboratory-grade equipment may not be feasible.

To validate the reliability of the fabricated spectrophotometer, a Wilcoxon Signed Rank test was performed to compare its measurements with those obtained using a standard UV-vis spectrophotometer. The test results revealed no significant difference between the two methods, confirming the accuracy and dependability of the fabricated device. This finding is of critical importance, as it demonstrates that the low-cost spectrophotometer can produce results comparable to a standard UV-vis spectrophotometer, which is widely recognized as the benchmark for precise protein quantification. Such comparability solidifies the fabricated spectrophotometer as a viable alternative for protein analysis, especially in resource-constrained environments or field-based applications.

A particularly valuable aspect of this outcome is the potential impact on rural or low-income regions where access to sophisticated laboratory facilities and expensive analytical tools is limited [32]. Goat milk is a significant staple dietary source in many developing countries, often providing essential nutrients to local populations.

The ability to accurately measure its protein content using an affordable and portable device enhances opportunities for better quality control and assurance, ensuring that consumers receive milk with optimal nutritional value [31]. This capability is especially beneficial for small-scale dairy farmers, cooperatives, and local milk producers who may lack the financial resources to invest in advanced laboratory equipment but still require reliable testing to maintain product quality and meet regulatory standards.

Furthermore, the success of the fabricated spectrophotometer in analyzing protein in a complex matrix like goat milk highlights its broader potential across various biological and agricultural applications. Accurate protein quantification is vital not only in food science but also in areas such as veterinary diagnostics, crop science, and environmental monitoring [33]. For instance, the device could be adapted to measure protein content in animal feeds, agricultural products, or even water samples, expanding its utility beyond the dairy industry. This adaptability underscores the device's potential to transform how biochemical analyses are conducted in low-resource settings.

In addition to its functionality, the ease of fabrication and operation of the spectrophotometer enhances its appeal. Its affordability, portability, and straightforward design make it accessible to a wide range of users, including students, researchers, and small-scale industries. These attributes could encourage the incorporation of the spectrophotometer into educational curricula, enabling students and researchers in underserved regions to engage in hands-on biochemical analyses that were previously beyond their

Table 5. Comparison of the fabricated spectrophotometer with alternative low-cost spectrophotometric methods

Principle/Sensor	Light source	Wavelength (nm)	Target analyte	Linear range (ppm)	LOD (ppm)	Cost	Remarks	Ref
TSL2591 light sensor, Arduino	750 nm LED	750	Protein (Casein, Lowry Method)	100–700	18.05	~USD 20	High precision and accuracy; validated with Wilcoxon test; open-source; 3D-printed case	This work
Smartphone camera + LED backlight	White LED	RGB analysis	Hemoglobin	NA	NA	Smartphone-based	Non-invasive; requires app and calibration; not specific to proteins	[20]
Digital camera (Paper Microzone)	Scanner light	Broad spectrum	Total Phenolics	20–500	4.35	Very low	Good for colored analytes; paper-based platform	[22]
Color sensor (TCS34725)	LED	~520 (green)	Glucose (Enzyme reaction)	25–200	~10	Low	Easy integration; suited for colorimetric enzyme assays	[25]
Office scanner + software	Flatbed scanner	RGB	Pesticides (Colorimetric chip)	50–1000	25	Low	Good for field use; limited real-time application	[24]
UV-vis + smartphone RGB	UV-vis + camera	400–800	Arsenic	10–500	1.2	Moderate	Dual mode detection; not designed for proteins	[6]
Hamamatsu C12880MA + Arduino Nano	White LED	450–750	Various	Broad spectrum	~0.1 AU	~EUR 225	Fully open-source, mobile/PC app, high resolution (15 nm), good for kinetic studies	[34]
Smartphone ALS (ambient light sensor)	Narrow-band LEDs	400–933	Proteins (e.g., BSA)	N/A	N/A	<€5	Very low cost, portable, educational use, customizable 3D-printed holder	[35]
Smartphone + App + 3D-printed enclosure	White light	VIS range	General lab use	N/A	N/A	~USD 15–30	For BYOD in labs; paired with Spectral Workbench software	[36]
STM32F4 + integrated colorimeter	Laser @880 nm	880	Phosphate (in water)	0.02–9.5 mg/L	0.009 mg/L	Moderate	Highly miniaturized; precise; microfluidic design; GSM/ZigBee connectivity	[37]
Smartphone camera (HSV/RGB) + magnetic textile extraction	LED	400–600 est.	Dyes (e.g., food colorants)	0.1–1.5 mg/L est.	~0.1 mg/L	Low	Used smartphone apps for color analysis; indirect quantification method	[38]

Note: Cost categories are approximate: Low (~< USD 50), Moderate (USD 50–300), High (>USD 1000). NA = Not available in the original study. The fabricated spectrophotometer stands out for its affordability, precision, validation through statistical testing, and real-world application in complex samples like milk. Smartphone based was not included in the smartphone price and software development cost

reach. The fabricated spectrophotometer aligns with global initiatives to promote equitable access to scientific tools and education by bridging the gap between traditional, high-cost analytical methods and modern, sustainable technologies.

To further emphasize the advantages of the fabricated spectrophotometer, a comparative analysis with previously reported low-cost spectrophotometric approaches is provided in Table 5. This comparison considers parameters such as sensing technology, light source, working wavelength, linear range, detection limit, cost, and analytical target. The proposed spectrophotometer demonstrates a favorable balance between affordability, sensitivity, and applicability. Unlike many camera-based or scanner-based alternatives, this device offers real-time digital readings, reliable calibration, and validated statistical performance in real sample analysis. The integration of an open-source microcontroller, 3D-printed housing, and compatibility with educational environments further enhances its potential for adoption in diverse settings [32].

■ CONCLUSION

This study presents a low-cost spectrophotometer using an Arduino microcontroller and TSL2591 light sensor for protein quantification via the Lowry method. The device, featuring a 750 nm LED and a 3D-printed case, achieved a linear range of 100–700 ppm ($R^2 = 0.9991$) with good precision (HORRAT 0.982). Results from goat milk samples showed no significant difference compared to a commercial UV-vis spectrophotometer, confirming its accuracy in real-sample analysis. Despite its performance, the device has limitations, including a relatively high LOD/LOQ and potential matrix effects in complex samples. While reconstituted milk was used without pretreatment, future improvements may address these sources of error. The Arduino code used to convert light intensity to absorbance is available upon reasonable request and is easily adaptable. This open-source and accessible platform offers a practical tool for biochemical analysis in educational, clinical, and low-resource environments, supporting broader adoption of affordable analytical technology.

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■ CONFLICT OF INTEREST

No conflict of interest to declare.

■ AUTHOR CONTRIBUTIONS

Conceptualization, writing—review and editing, Amin Fatoni, Dadan Hermawan, and Mekar Dwi Anggraeni; methodology, Amin Fatoni, Lestha Aurel Salsabilla, and Mekar Dwi Anggraeni; validation, Lestha Aurel Salsabilla, Amin Fatoni, Dadan Hermawan, Hartiwi Diastuti, and Mekar Dwi Anggraeni; formal analysis, investigation, funding acquisition, Lestha Aurel Salsabilla, Amin Fatoni, and Dadan Hermawan; resources, Amin Fatoni, Hartiwi Diastuti, and Mekar Dwi Anggraeni; data curation, visualization, writing—original draft preparation, Lestha Aurel Salsabilla, and Amin Fatoni; supervision, Amin Fatoni and Dadan Hermawan; project administration, Hartiwi Diastuti, and Mekar Dwi Anggraeni. All authors have read and agreed to the published version of the manuscript.

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