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Diagnostic Value of Error Analysis in Undergraduate Biochemistry Laboratory Education

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Abstract

Monitoring and evaluating students' analytical rigor in the biochemistry laboratory is crucial for fostering higher-order thinking skills. However, systematic assessment of their error analysis abilities is rare. This study aimed to evaluate undergraduate students' laboratory competencies using a cross-sectional comparative research design focused on protein quantification. Eleven different animal-based food ingredients across four groups (meat, local freshwater fish, poultry eggs, and seafood) were analyzed in three biological replicates (total n = 33 experimental units). The core methodology was colorimetric quantification using the Biuret method via UV-Vis spectrophotometry at 540 nm. The resulting experimental data showed that the meat group yielded the highest extractable protein content (6.48 ± 0.45%), followed by local freshwater fish (5.02 ± 0.31%), poultry eggs (4.98 ± 0.28%), and seafood (2.42 ± 0.19%). The obtained protein content data were then statistically analyzed using the non-parametric Kruskal-Wallis test to determine whether there was a significant difference in protein values between the four groups ($p = 0.124$, $\eta^2 = 0.15$, indicating a small to medium effect size). Major limitations of this practical protocol were identified, including that the simple extraction method selectively isolates only the water-soluble fraction of sarcoplasmic proteins. This leads to systematic underestimation (up to 80-90% deviation) when compared to theoretical total protein literature standards. Methodologically, this study highlights the limitations of standard university extraction protocols. Pedagogically, these data reveal problematic facts that could form the basis for curriculum changes and recommendations for providing better diagnostic tools.

Keywords: biochemistry education, biuret method, error analysis, laboratory competency, protein quantification

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1. Introduction

Across the globe, science educators are increasingly challenged to prepare graduates who can apply scientific knowledge to address complex societal, environmental, and technological problems. Consequently, laboratory learning has gained renewed attention as a cornerstone of chemistry education due to its potential to promote higher-order thinking, experimental competence, and authentic scientific practices. In higher education settings, biochemistry laboratory courses, including those offered at Universitas Negeri Surabaya (Unesa), represent a key instructional context for nurturing these 21st-century scientific capabilities (Elawady, 2009). Modern science pedagogy dictates that undergraduate students must acquire advanced analytical proficiency, data interpretation skills, and rigorous error-analysis capabilities, rather than merely developing manual dexterity in handling apparatus (Nechypurenko et al., 2025). Protein quantification laboratories

occupy a central role in biochemistry instruction because they integrate disciplinary knowledge with the practical competencies required for scientific investigation. By engaging students in measuring and interpreting biomolecular concentrations, these activities foster conceptual understanding of protein chemistry while supporting the development of analytical reasoning, quantitative data literacy, and laboratory problem-solving skills essential to contemporary chemistry education (Gray et al., 2015). However, traditional laboratory instructional designs frequently devolve into "cookbook" routines. When laboratory instruction is predominantly structured around reproducing idealized outcomes, students may become passive implementers of experimental protocols rather than active investigators of scientific phenomena. This emphasis on procedural compliance can restrict opportunities to evaluate sources of experimental variation, interrogate unexpected findings, and construct evidence-based explanations, thereby limiting the development of critical thinking and scientific reasoning skills that are central to contemporary chemistry education (Goodey & Talgar, 2016).

The Biuret assay remains a cornerstone pedagogical choice in undergraduate biochemistry laboratories owing to its operational simplicity, cost-effectiveness, and excellent reproducibility (Harvey and Ferrier, 2011). The analytical principle underlying the Biuret assay provides a meaningful connection between chemical theory and experimental practice. Under alkaline conditions, Cu^{2+} ions coordinate with peptide bond nitrogen atoms to form a purple-colored complex whose absorbance can be quantitatively measured at 540 nm using UV–Vis spectrophotometry. This reaction not only enables reliable protein determination but also serves as an instructional platform through which students can apply concepts of coordination chemistry, structure–property relationships, and quantitative spectroscopic analysis to authentic biochemical investigations (Dahal, 2024). Despite the simplicity of the subsequent spectrophotometric measurements, the quantitative accuracy of the entire assay is highly dependent upon the initial sample processing and extraction efficiency. When complex solid animal tissues—such as meat, fish, eggs, and seafood—are processed using only distilled water as the solvent, a profound biochemical limitation arises. This simple aqueous extraction isolates only the water-soluble sarcoplasmic protein fraction, leaving the bulk of myofibrillar and stroma structural proteins entrapped within the discarded cell matrix debris (Tornberg, 2005).

This structural constraint underscores a critical research gap at the intersection of analytical chemistry and science education. Although an abundance of literature documents the total nutritional composition of animal proteins or explores sophisticated extraction mechanisms for industrial applications, educational research rarely investigates how undergraduate students handle systematic data discrepancies in a practical environment. In typical university laboratory settings, when students obtain extractable protein percentages that are 50% to 90% below the total protein values listed in reference databases due to these extraction limitations (Soladoye et al., 2025), they commonly attribute the deviation to personal operational incompetence or pipetting failures. Consequently, very few studies have intentionally utilized these method-dependent anomalies as a deliberate pedagogical instrument to assess and enhance students' critical thinking and methodological literacy.

This study responds to the growing call for laboratory instruction that simultaneously promotes scientific knowledge acquisition and the development of authentic scientific practices. Its primary novelty lies in the integration of biochemical content investigation and competency-based laboratory assessment within a single experimental design. Specifically, the study not only compares extractable protein yields from diverse animal-based matrices using the Biuret assay but also leverages experimental inconsistencies and measurement deviations

as learning opportunities to evaluate students' analytical reasoning, technical proficiency, and error-analysis capabilities. By conceptualizing laboratory error as a productive component of scientific inquiry rather than a procedural deficiency, the study offers a new pedagogical perspective for designing chemistry laboratory experiences that more closely reflect the realities of scientific research and evidence-based problem solving.

2. Research Method

2.1. Matrix Selection and Sampling Strategy

The experimental matrices comprised 11 varieties of animal-derived protein sources, systematically classified into four core analytical cohorts: meat tissue (chicken, beef, and goat), local freshwater aquaculture fish (catfish, tilapia, and milkfish), poultry egg components (whites of free-range chicken, commercial chicken, and quail eggs), and marine invertebrate seafood (shrimp and blood clams). To maximize sampling representativeness and mitigate statistical bias, all raw materials were acquired in biological triplicates from three distinct traditional and retail marketplaces in Surabaya, Indonesia. Chemical procedures utilized analytical-grade reagents throughout, specifically the Biuret reagent (Merck; stabilized at pH 12.5 to ensure uniform copper-peptide coordination), crystalline Bovine Serum Albumin (BSA) standard (Merck, ≥96% purity), and deionized water (Chemindo) as the solvent phase.

2.2. Instrumental Parameters and Quality Control

Colorimetric measurements were executed using a double-beam UV-Vis Spectrophotometer, sweep-monitored within a spectral range of 540–560 nm, with quantitative data logged at peak absorption (540 nm) (Shen, 2023). To minimize systemic laboratory errors and maintain internal quality control, all volumetric pipettes, mechanical vortex instruments, and glassware underwent rigorous verification and calibration protocols prior to starting the experimental trials (World Health Organization, 2011). Centrifugal separations were conducted utilizing a benchtop centrifuge at a regulated temperature.

2.3. Sample Processing and Aqueous Extraction Limits

To establish an empirical baseline for homogenization, a 10.0-gram portion of each biological triplicate was thoroughly pulverized using a standardized ceramic mortar and pestle to achieve matrix uniformity. From this processed mass, a 1.0-gram sub-sample was accurately isolated via an analytical balance. The aliquot was immediately suspended in 10.0 mL of deionized water and mechanically vortexed for exactly 2 minutes to homogenize the suspension. The slurry was subsequently spun at 3500 rpm for 10 minutes at 25°C. The resulting supernatant fluid, which contained exclusively the extractable water-soluble protein fraction, was meticulously decanted into clean test tubes for immediate spectrophotometric analysis, while the insoluble myofibrillar and stroma pellet was separated.

2.4. Assay Validation and Calibration Curve Modeling

The analytical integrity of the colorimetric assay was verified through strict methodology parameters. A reference stock solution of BSA (10.0 mg/mL) was sequentially diluted with deionized water to construct a five-point working standard curve (1.0, 2.0, 3.0, 4.0, and 5.0 mg/mL). For color development, a 1.0 mL volume of each working standard was combined with 5.0 mL of the alkaline Biuret reagent, mixed thoroughly, and incubated at 37°C for 10 minutes to reach reaction equilibrium. Methodological acceptance was strictly bound to the following validation profile: (1) Linearity: The linear regression model ($y = mx + c$) was accepted for sample quantification only if the coefficient of determination reached $R^2 \geq 0.990$. (2) Sensitivity Thresholds: The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated mathematically based on the standard deviation of the reagent blank response (σ) and the response slope (S), applying the standard equations:

$$LOD = (3.3 \times \sigma) / S$$
$$LOQ = (10 \times \sigma) / S$$

(3) Control Assays: Negative control batches (deionized water blank + reagent) and positive controls (known BSA control points) were ran simultaneously alongside every sample batch.

2.5. Quantitative Absorbance Measurement

For sample analysis, a 1.0 mL portion of the isolated aqueous supernatant was added to 5.0 mL of the alkaline Biuret reagent. The matrix was incubated under identical thermal conditions (37°C for 10 minutes) to complete the chelation reaction. Absorbance values were collected at 540 nm against the prepared negative control blank. The quantitative data points were then calculated against the validated standard curve equation to determine the concentration of extractable protein.

2.6. Statistical Analysis

All extraction procedures and spectrophotometric readouts were performed in technical triplicates for each biological replicate, generating a total of n = 33 distinct experimental data points. Intra-laboratory precision was monitored via the Coefficient of Variation (CV%). Because the sample distribution within individual dietary subsets did not meet the sample size requirements for parametric tests, the assumptions for One-Way ANOVA were violated. Consequently, the statistical significance of differences in extractable protein values across the four food cohorts was determined via the non-parametric Kruskal-Wallis test, using a confidence threshold of $p < 0.05$. Statistical computations were processed via SPSS software.

3. Result and Discussion

3.1. Standard Calibration Curve Validation

To confirm the reliability of the colorimetric assay, the validation metrics of the Biuret calibration curve generated by the undergraduate cohorts were systematically monitored. The high coefficient of determination obtained from the calibration model reflects the overall quality of the experimental procedure and supports the reliability of the generated analytical data. From an educational perspective, this outcome indicates that students successfully applied fundamental laboratory practices, including accurate dilution, precise pipetting, and careful instrument operation. Such competencies are critical for ensuring data validity in quantitative biochemical analyses and represent important indicators of laboratory proficiency in chemistry education (Sonawane et al., 2019). The calibrated standard curve equation was established as $y = 0.1245x + 0.012$, operating within a validated working range of 1.0 to 5.0 mg/mL of Bovine Serum Albumin (BSA). The optical response exhibited excellent linearity, with a coefficient of determination (R^2) of 0.994 ± 0.002, consistently exceeding the standard validation baseline of ≥0.990 (Harvey and Ferrier, 2011). Additionally, the calculated Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined to be 0.15 mg/mL and 0.45 mg/mL, respectively. These sensitivity thresholds prove that the spectrophotometric setup possessed sufficient resolution to detect low concentrations of proteins within the isolated fluids.

3.2. Quantitative Protein Profiles and Method-Dependent Deviation

The quantitative evaluation of protein levels across the 11 animal-derived matrices demonstrated highly reproducible data points within student cohorts, yet revealed a severe negative bias when compared with theoretical total protein standards. The empirical values, expressed as Mean ± Standard Deviation (SD) alongside the Coefficient of Variation (CV%), are structured in Table 1.

Table 1. Analytical Comparison of Experimental Extractable Protein and Theoretical Nutritional Baselines

Sample Category	Spesific Sample Material	Experimental Extractable Protein (% ± SD)	Intra-Assay Precision (CV%)	Theoretical Total Protein (%)	Methodological Interpretation & Reference
Meat	Chicken breast	8.73 ± 0.42	4.81%	~ 21.0 - 23.0	Sarcoplasmic fraction isolation (Felayati, 2019)
	Beef loin	7.95 ± 0.35	4.40%	~ 20.0 – 22.0	Sarcoplasmic fraction isolation (Bowker et al., 2012)
	Goat meat	2.77 ± 0.18	6.50%	~ 19.0 – 21.0	Restricted sarcoplasmic yield (Setiawan, 2014)
Fish	Catfish	9.02 ± 0.51	5.65%	~ 17.0 – 18.0	High moisture extraction (Heri, 2019)
	Tilapia	4.78 ± 0.29	6.07%	~ 16.0 – 19.0	Sarcoplasmic matrix dissolution (Hasyim et al., 2020)
	Milkfish	1.27 ± 0.08	6.30%	~ 20.0 – 24.0	Extreme structural trapping (Hafiludin, 2015)
Poultry Eggs	Free-range egg white	4.90 ± 0.31	6.33%	~ 10.0 – 11.0	Incomplete macromolecular breakdown (Bakhtra et al., 2016)
	Commercial egg white	4.07 ± 0.22	5.41%	~ 10.0 - 11.0	Viscosity-driven diffusion limits (Bakhtra et al., 2016)
	Quail egg white	5.97 ± 0.38	6.37%	~ 11.0 – 12.0	High water-soluble protein density (Bakhtra et al., 2016)
Sea food	Fresh shrimp	1.65 ± 0.11	6.67%	~ 18.0 – 20.0	Connective matrix interference (Inthe et al., 2023)
	Blood clams	3.18 ± 0.20	6.29%	~12.0 – 14.0	Glycogen and moisture suppression (Inthe et al., 2023)

3.3. Chemical Critique of Extraction Constraints and Protein Loss

The pivotal discovery of this investigation is the identification of a severe negative bias, with experimental figures ranging from 80% to 95% beneath the theoretical figures outlined in nutritional databases. Instead of reflecting student inadequacy, this variability linked to methodology can be entirely attributed to the intrinsic chemical constraints of the extraction technique. Proteins contained within solid animal muscle tissue can be categorized into three separate solubility groups: sarcoplasmic (water-soluble, making up approximately 30% of total muscle), myofibrillar (salt-soluble, accounting for 50–60%), and stroma (insoluble connective tissues, representing 10–20%) (Tornberg, 2005).

Due to the educational curriculum's requirement to strictly utilize deionized water without modifications to ionic strength or pH, students isolated solely the water-soluble sarcoplasmic fraction (which includes myoglobin and metabolic enzymes). The relatively low extraction efficiency observed in muscle-derived samples can be attributed to the physicochemical properties of the dominant myofibrillar proteins, particularly actin and myosin, which constitute a

substantial proportion of muscle tissue. These structural proteins exhibit limited solubility in aqueous environments and generally require elevated ionic strength (e.g., >0.5 M NaCl) or alkaline conditions to disrupt intermolecular interactions and facilitate protein solubilization (Li et al., 2023). As a result, these prominent myofibrillar fractions remained ensnared within the cellular debris pellet and were removed during the post-centrifugation decantation process. Furthermore, the lack of a moisture content correction protocol indicated that the substantial water mass inherent in raw samples diluted the observable protein concentration, leading to reduced final percentages. This chemical fact fully accounts for the significant differences observed in beef (7.95% experimental compared to 22% theoretical) and milkfish (1.27% experimental versus 20% theoretical).

3.4. Pedagogical Reflection and Student Competency Diagnostics

Transitioning the focus from the sole analysis of food composition to the realm of chemistry education, this experimental discrepancy acts as an effective diagnostic instrument to assess student lab proficiency. Undergraduate achievement was methodically assessed across five fundamental educational foundations: (1) Pipetting Precision and Calibration Literacy: The student cohorts demonstrated significant technical mastery over multi-stage serial dilutions and micropipette operations by achieving a very linear calibration curve (R^2 = 0.994). (2) Replicated Analytical Consistency: Students maintained strong mechanical consistency throughout technical and biological triplicates, as seen by the minimal intra-assay variance (all CV% values stayed below the 7% threshold). (3) Higher-Order Thinking Skills (Advanced Error Analysis): Instead of making adjustments to their data when faced with data that was significantly "lower than the textbook," students were required to perform a methodological critique. Although the extraction solvent (water) limited the analytical scope to sarcoplasmic fractions, the students were able to determine that their operating technique was perfect. As a result, they were able to change from being passive recipe-followers to reflective analytical chemists. (4) Data Reporting and Scientific Communication: By moving from reporting unprocessed raw absorbances to creating statistical summaries with mean, SD, and variance values, students showed a notable improvement in data presentation. (5) Curricular Time Management: Students demonstrated outstanding operational efficiency by completing intricate multi-sample extractions, colour development, and multi-wavelength spectrophotometric monitoring within a typical 150-minute laboratory window.

To place these results within Unesa's broader academic community, a structured practical assessment rubric was created (Table 2) to formalize how error analysis may be integrated into future biochemistry practical curricula.

Table 2. Proposed Pedagogical Rubric for Critical Error Analysis in Biochemistry Practicums

Competency Domain	Basic (Score 1-2)	Proficient (Score 3)	Advance (Score 4)
Technical Execution	Inconsistent pipetting: R^2<0.95	Precise pipetting: R^2≥0.99, high CV%	Flawless technique; R^2≥0.99, CV%<7%
Data Interpretation	Accepts raw data blindly without theoretical comparison	Identifies deviations from theory but labels them as "personal mistakes"	Correctly correlates deviations with chemical extraction and solubility constraints
Methodological Critique	Unable to explain limitations of the Biuret assay	Understands the basic Biuret mechanism but misses extraction variables	Critically evaluates sample interference, moisture bias, and solubility profiles

The structural rubric outlined in Table 2 provides a concrete framework for transforming the biochemistry practical from a passive technical exercise into an inquiry-driven learning environment. By establishing clear thresholds for error analysis, this rubric shifts the definition of student success; an "Advanced" designation is no longer reserved for students who happen to achieve idealized textbook numbers, but rather for those who can scientifically evaluate why deviations occur.

Imbedding this critical error literacy into the biochemistry curriculum at Universitas Negeri Surabaya (Unesa) carries significant pedagogical implications (Nechypurenko et al., 2025). The predominance of verification-oriented laboratory activities in chemistry education often obscures the inherently uncertain nature of scientific experimentation. Consequently, students may have limited experience in confronting anomalous results, identifying sources of error, and constructing evidence-based explanations, thereby restricting the development of the analytical and problem solving capabilities required for authentic scientific inquiry (Kruse et al., 2022). When students encounter a 95% protein loss due to method constraints, they are forced to confront the boundary between theoretical ideals and practical chemistry. This active cognitive dissonance bridges the gap between mechanical execution and deep conceptual understanding. Ultimately, using systematic laboratory anomalies as a diagnostic learning asset prepares undergraduate students for future scientific research, where anomalies are not failures to be hidden, but discoveries to be methodologically investigated.

4. Conclusion

To investigate broader analytical trends, individual matrices were compiled into their respective broad dietary categories. The meat group displayed the highest mean extractable protein yield (6.48 ± 0.45%), followed sequentially by local aquatic fish (5.02 ± 0.31%), poultry eggs (4.98 ± 0.28%), and marine seafood (2.42 ± 0.19%). To accommodate the small sample sizes within specific subsets, a non-parametric Kruskal-Wallis test was conducted to maintain statistical stringency. The analysis indicated that the differences in extractable protein fractions among the four major dietary groups were not statistically significant ($H(3) = 5.75$, $p = 0.124$). However, the partial eta-squared calculation yielded a value of $\eta^2 = 0.15$, indicating a substantial educational effect size. This implies that while biological variances exist, the structural boundaries of the laboratory protocol uniformized the final empirical outcomes, a phenomenon often observed when standardization overrides matrix diversity in undergraduate practicals (Nechypurenko et al., 2025).

The primary limitation of this research stems from the structural setup of the undergraduate curriculum, which excluded parallel non-aqueous extractions and moisture correction steps, thereby limiting the data's utility for comparative food chemistry reporting. To enhance the biochemistry curriculum, it is highly recommended that university laboratory courses transition from routine, recipe-following designs toward inquiry-driven models. Specifically, future modules should introduce parallel extraction paths that compare aqueous extraction directly against alkaline (0.1 M NaOH) or detergent-based (SDS) extraction systems. Implementing these changes will provide undergraduate students with clear, evidence-based exposure to protein solubility behaviors, significantly improving both their technical laboratory precision and their higher-order critical thinking skills within the scientific community.

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