

HIGH-YIELDING TOTAL RNA ISOLATION OF CHICKEN LIVER USING LITHIUM CHLORIDE-BASED PURIFICATION METHOD FOR POLYSACCHARIDE-RICH TISSUES

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ABSTRACT

The fidelity of transcriptome profiling and the subsequent construction of reliable libraries for gene expression analysis are contingent upon the quality of isolated RNA. While high-yielding extraction protocols for plant tissues are well established, current methods can be less efficient for animal tissues, particularly those polysaccharide- and metabolite-rich samples, such as the liver. Commercial kits often provide solutions but are costly, limiting accessibility in resource-constrained laboratories. This study reports an optimized total RNA isolation protocol using lithium chloride (LiCl)-based purification to address these limitations. The method effectively eliminated glycogen co-precipitation and contamination through stepwise purification. Comparative analysis demonstrated that RNA purity (2.15 OD ratio) and yield (150.45 ng/μL) were statistically comparable to conventional and spin-column methods ($p = 0.011$ and 0.004 , respectively). The RNA isolates demonstrated suitability for downstream molecular applications, as confirmed by successful cDNA synthesis and PCR amplification of the β -actin housekeeping gene. By combining efficiency, reproducibility, and affordability, this LiCl-based protocol offers a broadly applicable approach for RNA isolation.

Keywords: cost-effective RNA extraction, lithium chloride-based RNA purification, glycogen contamination, transcriptome profiling

INTRODUCTION

Analysis of gene expression is fundamental to understanding cellular processes and biological functions. Techniques such as RNA sequencing (RNA-Seq) (Newton *et al.*, 2020) and RT-qPCR arrays (Sakyi *et al.*, 2023) rely on high-quality RNA extraction for accurate results. However, existing methods for RNA extraction present limitations. Traditional approaches involving phenol/chloroform or guanidinium thiocyanate suffer from contamination issues, safety concerns, and potential for incomplete phase separation (Tavares *et al.*, 2011). Commercial kits such as TRIzol reagents and column-based kits (Sharma *et al.*, 2019) offer convenience but are costly and less suitable for large sample volumes (Yoshino *et al.*, 2020).

RNA isolation from tissues rich in polysaccharides and secondary metabolites, such as liver, is particularly difficult because polysaccharides co-precipitate with RNA, reduce purity, and inhibit enzymatic reactions required for downstream applications (Sharma *et al.*,

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2019; Gautam, 2022). This biochemical interference underlies the hypothesis that a selective precipitation method can improve RNA recovery from such tissues.

The high cost of advanced transcriptome analysis further restricts research in resource-limited settings. Therefore, the objective of this study is to develop an optimized, cost-effective total RNA isolation protocol using lithium chloride (LiCl)-based purification. By addressing polysaccharide contamination and glycogen co-precipitation, this method aims to provide a broadly applicable solution for reliable RNA extraction across diverse tissue types.

MATERIALS AND METHODS

Liver Tissue Collection

Liver tissue samples were collected from 9-week-old Banaba chickens at a commercial slaughterhouse in Los Baños, Laguna. All tools used for tissue dissection were sterilized and autoclaved at 121°C for 60 minutes beforehand to prevent contamination with exogenous RNases. To preserve RNA integrity, tissue samples were immediately snap-frozen in liquid nitrogen following collection and subsequently stored at -80 °C in a biofreezer, ensuring stabilization of nucleic acids until further processing.

Homogenization and Tissue Disruption

Frozen liver samples were thawed and finely minced on a pre-chilled Petri dish containing ice-cold 1X phosphate-buffered saline (PBS) supplemented with KCl (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.47 mM KH₂PO₄, pH 7.4) using sterile scalpels and scissors. Liver tissue samples weighing ≤100 mg (≤0.1 g) were pulverized in liquid nitrogen using a sterilized, pre-chilled mortar and pestle. A sterile stainless-steel spoon precooled in liquid nitrogen was then used to transfer the powdered tissue to pre-chilled, 1.5 mL RNase-free microcentrifuge tubes.

Total RNA Isolation

Optimized SDS/LiCl-based Total RNA Isolation

The optimized SDS/LiCl-based RNA isolation method used in this study was adapted from Vennapusa *et al.* (2020) for plant tissues and modified for chicken liver tissue, with 3 biological replicates performed. About 100 mg of powdered liver tissue was transferred to pre-chilled RNase-free microcentrifuge tubes. Afterward, 600 µL of autoclaved RNA extraction buffer, composed of 100 mM Tris-HCl, 2.5% PVP, 25 mM EDTA 2Na, 2.5 M NaCl, UltraPure™ DNase/RNase-Free Distilled Water, and 2.5% β-mercaptoethanol, was added to the sample, then mixed thoroughly. If the tissue did not dissolve completely, the buffer volume was increased to 1 mL. The homogenate was allowed to incubate at ambient temperature for 5 minutes. Subsequently, 60 µL of 20% SDS was added to reach a final concentration of 2%, after which the tubes were vortexed and incubated again at room temperature for 2 minutes.

The homogenized samples were centrifuged at 15,890 × g for 5 minutes at 4 °C. An equal volume (approximately 600 µL) of phenol solution saturated with 0.1M citrate buffer, mixed with chloroform and isoamyl alcohol in a 25:24:1 ratio, was subsequently added to

each preparation. The mixture was then vortexed thoroughly. Following this, centrifugation was performed at $15,890 \times g$ for 5 minutes at 4°C . The upper aqueous layer containing RNA was carefully collected, ensuring that the lower phases with proteins, polysaccharides, and other organic compounds remained undisturbed.

Chloroform (200 μL) was added to the collected aqueous phase, followed by thorough mixing using a vortex mixer. The mixture was centrifuged at $15,890 \times g$ for 5 minutes at 4°C , after which the upper aqueous fraction was carefully collected once more. To reach a final concentration of 2 M, approximately 160 μL of 8 M LiCl was added. Additionally, 1/10 volume of 3 M sodium acetate (pH 4.8, 60 μL) was introduced. The sample was mixed gently by inverting it 4 to 6 times. Subsequently, the sample was incubated at -20°C for 15 minutes to facilitate RNA precipitation. Following incubation, centrifugation was performed at $15,890 \times g$ for 5 minutes at 4°C .

After discarding the supernatant, the clear pellet was rinsed with 500 μL of 2 M LiCl and subjected to centrifugation at $15,890 \times g$ for 5 minutes at 4°C . The supernatant was then decanted. The pellet was further washed with pre-chilled 80% ethanol using the same centrifugation parameters. Finally, the wash solution was carefully decanted, and any residual ethanol was allowed to evaporate under controlled airflow conditions in the hood to prevent solvent carryover and ensure RNA integrity. The RNA pellet was allowed to dry completely (avoiding over-drying). The dried RNA pellet was dissolved in an appropriate volume (30-50 μL) of UltraPure™ DNase/RNase-Free Distilled Water. The purified RNA was then stored at -80°C (biofreezer).

Conventional Total RNA Isolation

The conventional method utilized the SDS/Phenol extraction, following a procedure similar to the optimized method, with 3 replicates performed. An extraction buffer containing 100 mM Tris/HCl, 0.1 M NaCl, 1% SDS, 25 mM EDTA, 2% PVP-40, and 2% β -mercaptoethanol was prepared with UltraPure™ RNase-free water and autoclaved. Unlike the optimized method, it did not include the two-phase purification with 2 M LiCl. The extracted RNA was then stored in a biofreezer at -80°C .

Total RNA Isolation Using Commercial Kit

Total RNA was extracted using the PureLink™ RNA Mini Kit (Thermo Fisher Scientific) in accordance with the manufacturer's instructions for small tissue samples (≤ 100 mg), with three independent replicates performed. Tissue samples were transferred to 1.5 mL microcentrifuge tubes and treated with 600 to 1000 μL of Lysis Buffer containing β -mercaptoethanol. Following homogenization with an 18-gauge needle and an RNase-free syringe, the samples were centrifuged at $12,000 \times g$ for 2 minutes. The supernatants were transferred to new microcentrifuge tubes.

Each sample received one volume of chilled 70% ethanol, was vortexed, and up to 700 μL was loaded onto spin cartridges. Cartridges were centrifuged at $12,000 \times g$ for 15 seconds, and the flow-through was discarded. This step was repeated until the entire sample had been processed. Sequential washes were then performed using Wash Buffer I and II, each followed by centrifugation at $12,000 \times g$ for 15 seconds. Finally, a 1-minute spin at $12,000 \times g$ was carried out to dry the cartridges before transferring them into recovery tubes.

For the elution step, 30–100 μL of UltraPure™ DNase/RNase-Free Distilled Water was added to each cartridge, followed by incubation and centrifugation at $\geq 12,000 \times g$ for

2 minutes. The resulting purified RNA was subsequently stored in a biofreezer at -80°C .

Qualitative and Quantitative Evaluation of the Extracted Total RNA UV-Vis Spectroscopy

The BioTek Epoch 2 Microplate Spectrophotometer (Massachusetts, USA) was utilized to evaluate both the concentration and purity of the extracted RNA. Immediately after extraction, 2 μL of each sample were analyzed to determine absorbance ratios at 260/280 nm and concentration ($\text{ng}/\mu\text{L}$). These measurements were crucial for calculating the volume needed for subsequent cDNA synthesis. An optical density of 1 at 260 nm corresponds to approximately 40 $\mu\text{g}/\text{mL}$ of single-stranded RNA, with pure RNA generally exhibiting an A260/A280 ratio close to 2.0 (≥ 2.0). Lower absorbance ratios indicate contamination, typically from proteins or phenolic compounds that exhibit strong absorption near 280 nm.

Agarose Gel Electrophoresis

The integrity of the extracted total RNA was assessed using 1% and 1.5% agarose gel electrophoresis. The samples underwent electrophoresis twice: first to confirm the presence of total RNA and then to verify PCR products from synthesized cDNA samples. To prepare the gel, 1X TAE buffer was made by diluting aliquots of a 50X Tris-acetate EDTA stock with distilled water. Agarose D1 Low EEO (Condalab) was dissolved in the buffer, microwaved until clear, and poured into a gel mold with a comb inserted. After cooling and comb removal, the gel was submerged in 1X TAE buffer in a gel box. A DNA ladder was prepared by mixing VC 100 bp Plus DNA ladder (Vivantis) with DNA loading dye (BlueJuice™ Gel Loading Buffer with GelRed® Nucleic Acid Gel Stain) and water. Samples mixed with loading dye were loaded into wells, with the ladder in the first well, samples in subsequent wells, and a negative control in the last.

The Mupid® One Electrophoresis System (Seraing, Belgium) was run at 100V for 30 minutes to verify total RNA presence and at 50V for 60 minutes to visualize PCR amplicons. The gel was then immediately exposed to UV light using the ENDURO™ GDS Touch Gel Documentation System (Tewksbury, MA, USA) to visualize bands of 18S and 28S rRNA and PCR amplicons, respectively.

Complementary DNA (cDNA) Synthesis

Complementary DNA (cDNA) was synthesized from the previously isolated total RNA using the TaqMan™ Reverse Transcription Reagents (Thermo Fisher Scientific). This process was conducted using an Applied Biosystems™ Veriti™ 96-Well Thermal Cycler (Massachusetts, USA). Enzymes were thawed from storage at -20°C , kept on ice, mixed, and briefly centrifuged to ensure proper mixing. A master mix for reverse transcription (RT) was prepared by combining specified components in each reaction tube (final volume of 20 μL) (Table 1).

Next, 50 μM random hexamers and the RNA template were added to each reaction tube containing the RT master mix. After capping and thorough mixing, tubes were briefly centrifuged to gather contents at the bottom. The cycling parameters recommended in the manufacturer's protocol (Table 2) were followed for the random hexamer reaction. Finally, the RT reactions were either immediately utilized for downstream applications or stored at -20°C .

Table 1. Components of TaqMan™ Reverse Transcription Master Mix*.

Component	Initial Concentration	Final Concentration	1X = 20 µL	10X = 200 µL
RNase-Free Water	—	—	to 20 µL	to 200 µL
RT Buffer	10X	1X	2.0 µL	20 µL
MgCl ₂	25 mM	1.75 mM	1.4 µL	14 µL
dNTP Mix	10 mM	0.5 mM each	4.0 µL	40 µL
DTT	100 mM	5.0 mM	1.0 µL	10 µL
RNase Inhibitor	20 U/µL	1.0 U/µL	1.0 µL	10 µL
MultiScribe™ RT	50 U/µL	2.5 U/µL	1.0 µL	10 µL
Random Hexamers	50 µM	2.5 µM	1.0 µL each tube	1.0 µL each tube (10 µL)
Template RNA	—	—	varies	varies

*Adapted from Life Technologies, 2014. User Guide: TaqMan Reverse Transcription Reagents, Protocol Pub. No MAN0009791 Rev A.0, www.documents.thermofisher.com.

Table 2. Thermal Cycling Parameters for Random Hexamers.

Primer Type	Temperature	Time
Random Hexamers	25°C	10 minutes
	37°C	30 minutes
	95°C	5 minutes
	4°C	Indefinitely

Gene Amplification and Polymerase Chain Reaction

For PCR amplification of the β-Actin (ACTB) gene product (128 bp), 2 µL of cDNA obtained from different RNA extraction methods was combined with a reaction mixture consisting of 1X TaqMan™ Universal PCR Master Mix (Thermo Fisher Scientific), 0.75 µM of the forward primer (ACTBF: CTGGCACCTAGCACAATGAA), 0.75 µM of the reverse primer (ACTBR: CTGCTTGCTGATCCACATCT), and nuclease-free water to a final reaction volume of 10 µL.

PCR amplification was carried out using the Applied Biosystems™ Veriti™ 96-Well Thermal Cycler (Massachusetts, USA) under optimized conditions: an initial denaturation at 95 °C for 5 minutes, followed by 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 52 °C, 55.2 °C, and 60 °C for 30 seconds (gradient optimization), and extension at 72 °C for 30 seconds. A final extension was performed at 72 °C for 5 minutes. Amplicons were analyzed by 1.5% agarose gel electrophoresis.

Cost and Statistical Analyses

The cost-effectiveness of the optimized total RNA isolation method was compared

against the conventional method and the commercially available PureLink™ RNA Mini Kit (Thermo Fisher Scientific). The total cost was calculated by adding the unit price of each reagent used in the protocol. Additionally, a cost-per-RNA yield or reaction (set at 50 reactions per method) was also computed, where the total cost was divided by the average RNA yield obtained from each method or 50 reactions for each method.

After data collection, ANOVA and post-hoc analyses in Jamovi Version 2.2 were conducted to determine significant differences in the purity and yield of isolated total RNA among the optimized method, conventional method, and commercial kit.

RESULTS AND DISCUSSION

Total RNA Isolation

The isolation of high-quality RNA from eukaryotic tissues and cells is critical for downstream applications such as next-generation sequencing (NGS)-based transcriptome analyses. However, this process can be particularly challenging for tissues rich in polysaccharides, such as the liver. In these samples, co-precipitation of polysaccharides with RNA can significantly compromise RNA yield and purity (Sharma *et al.*, 2019). The integrity and purity of total RNA extracted from chicken liver tissue samples were assessed using 1% agarose gel electrophoresis. The results showed intact 28S and 18S ribosomal RNA bands in RNA extracted using the optimized lithium chloride-based purification protocol (Figure 1: lanes 4-6), indicating minimal degradation. In contrast, the conventional SDS/Phenol method resulted in poor-quality RNA with indistinct rRNA bands, significant impurities in the gel wells, and a smeared background (Figure 1: lanes 1-3).

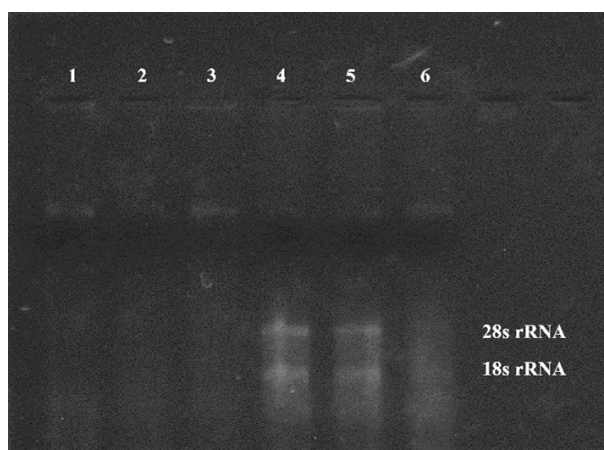


Figure 1. Agarose (1%) gel electrophoresis of total RNA isolates from chicken liver tissue. (Lanes 1-3: conventional method; Lanes 4-6: optimized method).

In assessing RNA purity using the lithium chloride-based purification method, A260/A280 absorbance ratios were measured with the BioTek Epoch 2 Microplate Spectrophotometer (Massachusetts, USA). Table 3 displays results showing that all RNA samples isolated using the optimized method had ratios above 2.0, indicating minimal contamination from proteins, solvents, and other substances. Conversely, the conventional

extraction method (used as a negative control) yielded lower ratios ranging from 1.459 to 1.860, suggesting significant contamination. The spin protocol (positive control) generally produced ratios around 2.0, with only one sample (G1) slightly below this threshold. Table 4 shows that the optimized method’s OD ratios were comparable to those of the total RNA isolates from the spin protocol, demonstrating the lithium chloride-based method’s consistently higher purity, as evidenced by all samples maintaining A260/A280 ratios above 2.0.

Table 3. Absorbance ratio (A_{260}/A_{280}) and concentration (ng/uL) of the isolated total RNA from chicken liver samples.

Method	Sample	A_{260}/A_{280} O.D. Value	Concentration (ng/uL)
Optimized	L1	2.163	177.588
	L2	2.129	136.505
	L3	2.143	137.253
Conventional	S1	1.860	29.953
	S2	1.459	11.026
	S3	1.676	30.556
Spin Protocol	G1	1.994	52.202
	G2	2.050	79.168
	G3	2.011	73.634

Table 4. Mean ± SD of O.D. values and concentration of the isolated total RNA using the optimized method (OM), conventional method (CM), and spin protocol (GS, kit-based method).

	Treatment	Mean	SD	p-value
A_{260}/A_{280} O.D. Values	OM	2.15 ^a	0.0171	0.011*
	GS	2.02 ^a	0.0287	
	CM	1.67 ^b	0.2007	
Concentration (ng/uL)	OM	150.45 ^a	23.5063	0.004*
	GS	68.33 ^b	14.2427	
	CM	23.84 ^c	11.1057	

Values with the same superscripts are not significantly different; *p< 0.05

The A260/A280 ratio serves as a crucial indicator of nucleic acid purity, with a value around 2.0 or higher indicating minimal contamination by proteins and other impurities that absorb near 280 nm. The consistently high ratios observed in our samples emphasize the effectiveness of the lithium chloride-based RNA extraction method in generating clean total RNA suitable for downstream applications like qPCR and RNA sequencing. This purity

is essential for ensuring accurate and reliable results. The optimized method yielded an average RNA concentration of 150.449 ng/ μ L, with values ranging from 136.505 to 177.588 ng/ μ L. In contrast, the conventional organic method produced a markedly lower average of 23.845 ng/ μ L (range: 11.026–30.556 ng/ μ L), while the spin-column protocol yielded 68.335 ng/ μ L (range: 52.202–79.168 ng/ μ L). The p-value of 0.004 indicates that the optimized method was statistically comparable to both conventional and spin-column methods, yet with significant differences in concentration ($p < 0.05$) (Tables 3 and 4).

Comparing these values reveals that the optimized method consistently yields higher concentrations of total RNA compared to both the conventional and spin protocol methods. This indicates the initial efficiency of the optimized method in isolating RNA from liver tissue samples. The quality and suitability of the isolated RNA for downstream applications were further assessed through several experimental steps. Initially, total RNA was converted into cDNA, followed by PCR amplification using β -actin gene-specific primers. The resulting PCR products were analyzed via 1.5% agarose gel electrophoresis, which demonstrated the efficient and comparable performance of the optimized method. The electropherograms exhibited distinct and intact bands at the expected 128 bp length (Figure 2), validating the high quality of RNA extracted using the optimized method.

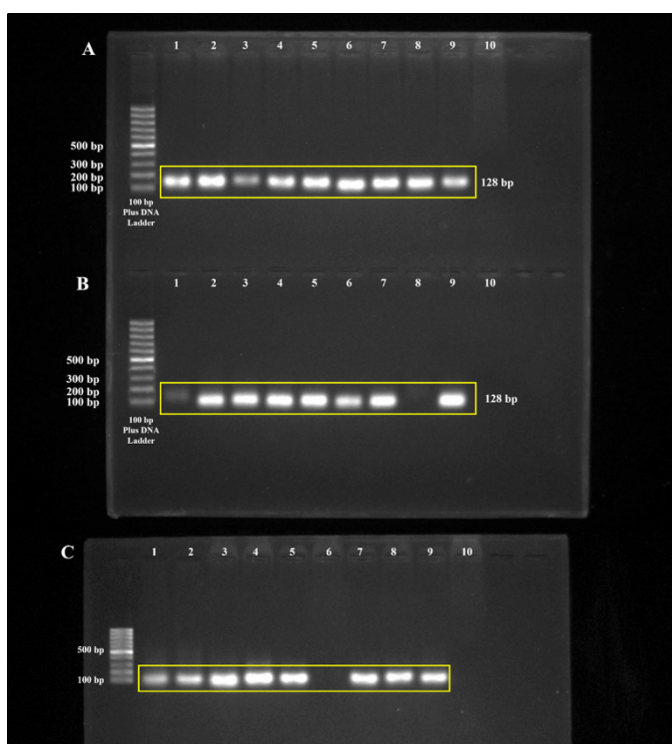


Figure 2. Agarose (1.5%) gel electrophoresis of total RNA isolated from chicken liver tissue samples. (Left lane: 100 bp Plus DNA Ladder (Vivantis); Lanes 1-3: conventional method; Lanes 4-6: optimized method; Lanes 7-9: spin protocol; Lane 10: negative control) (Annealing temperatures: A: 60°C; B: 55.2°C; C: 52°C).

Moreover, optimized annealing temperatures (52°C, 55.2°C, and 60°C) were found to yield distinct and solid bands of the desired length. Qualitative assessment of PCR products, particularly those amplified at an annealing temperature of 60 °C, demonstrated that the optimized protocol (lanes 4-6) consistently yielded more distinct bands compared with the conventional approach (lanes 1-3) and the spin-based method (lanes 7-9). The observed improvement suggests a reduction in nonspecific amplification, potentially leading to enhanced accuracy and reliability of downstream results. Furthermore, the consistent band intensity across replicates in the optimized lanes signifies tighter control over experimental variables. This stands in contrast to the variability observed in the conventional and spin protocol methods, which might be attributed to inconsistencies in reaction conditions or amplification efficiency.

However, discrepancies were noted in the results. Sample 8 (Lane 8: G2) at 55.2°C (Figure 2-B) and Sample 6 (Lane 6: S3) at 52°C (Figure 2-C) did not produce any visible band, possibly due to potential issues in sample preparation. Additionally, Lane 1 at 52°C displayed a very faint band (Figure 2-B), likely attributable to the low concentration of isolated RNA.

Lithium Chloride-based Purification Method

Next-generation sequencing (NGS) techniques, including poly(A) enrichment and library preparation, rely heavily on the quality and quantity of isolated RNA (Peimbert & Alcaraz, 2016). However, current RNA isolation methods often encounter challenges in obtaining sufficient RNA yields, particularly from complex animal tissues with high polysaccharide content. Existing protocols primarily optimized for plant samples leave a significant gap in the efficient isolation of high-quality RNA from animal tissues.

During total RNA isolation, the presence of a white, sticky, clumpy substance was observed post-centrifugation of samples treated with RNA extraction buffer. This substance indicates the presence of polysaccharides and glycogen within the sample, persisting throughout extraction despite washing and purification steps. This may impact the efficacy of the extraction buffer, contributing to lower absorbance ratios seen in conventional methods and reduced RNA concentrations obtained from both conventional and spin protocol approaches.

Vennapusa *et al.* (2020) optimized the conventional SDS-Phenol extraction method to isolate total RNA from starch-, protein-, and fiber-rich plant tissues. Modifications were introduced to the extraction buffer and RNA precipitation solutions specifically for polysaccharide-rich samples. The refined SDS-LiCl method outperformed existing methods, including commercial kits like TRIzol (Invitrogen) and the Plant RNeasy mini kit (Qiagen), as well as other established protocols such as the Furtado (2014) method and the CTAB-LiCl method (Song *et al.*, 2011). These modifications resulted in an efficient RNA extraction method yielding high-quality RNA. Gel electrophoresis revealed intense RNA bands, while spectrophotometric analysis showed A260/A280 ratios of ≥ 2.0 , indicating minimal contamination. The absence of starch co-precipitation enhanced RNA yield and quality, ensuring that the isolates were suitable for downstream applications such as RNA sequencing, transcriptome profiling, and gene expression analysis.

The optimized method in this study followed the same procedure as the conventional approach but with modified component concentrations for animal samples: NaCl (2.5 g), PVP (2.5 g), β -mercaptoethanol (2.5%), and SDS (2%). Higher concentrations of these

components promoted cell lysis, inhibited RNase activity, and prevented polysaccharide contamination (Wang *et al.*, 2012). To further purify the sample, an acidic phenol solution (pH 4.3) saturated with citrate buffer was applied, ensuring effective separation of RNA from remaining polysaccharide contaminants. RNA precipitation was markedly enhanced through the combined use of 2 M lithium chloride and one-tenth volume of 3 M sodium acetate (pH 4.8), consistent with the observations reported by Vennapusa *et al.* (2020). Incorporating the lithium chloride and sodium acetate into the step-wise precipitation promoted RNA yield, purity, and concentration compared to conventional methods. Sharma *et al.* (2019) further discussed that a sodium acetate and LiCl-based purification buffer improves RNA quality and allows additional removal of residual glycogen through reprecipitation. Similarly, Gautam (2022) demonstrated that lithium cations induce selective folding and precipitation of RNA, suggesting specific interactions that differ from those affecting DNA and proteins.

Cost Analysis

The cost-effectiveness of the enhanced total RNA isolation protocol was evaluated against both the conventional method and the commercially available PureLink™ RNA Mini Kit. For 50 reactions, the optimized method cost PHP 7,403.28, yielding 150.449 ng/μL RNA. Although the per-reaction cost was not numerically the lowest, the yield-to-cost ratio was more efficient. The conventional method cost PHP 6,072.17 but yielded only 23.845 ng/μL, while the spin protocol was the most expensive at PHP 282.32 per reaction with 68.335 ng/μL yield. Thus, when normalized to RNA yield, the optimized method was significantly more cost-efficient (Table 5).

Table 5. Cost analysis of the total RNA isolation methods.

Method	Average RNA Yield (ng/μL)	Total Cost (PhP)	Total Cost-per-RNA Yield (PhP)	Total Cost-per-Reaction (PhP)
Optimized Method	150.449	7,403.28	49.21	148.07
Conventional Method	23.845	6,072.17	254.65	121.44
Spin Protocol (kit)	68.335	14,116.06	206.57	282.32

^a at 50 total reactions

Scope and Limitations

This study was limited to chicken liver samples, a polysaccharide-rich tissue. While results demonstrate efficiency in this context, applicability to other tissues (e.g., cartilage, seeds, fungi, FFPE samples) remains to be validated. Additionally, only β-actin was tested as a marker gene; future studies will include low-expression genes to further assess sensitivity.

SUMMARY AND CONCLUSION

The lithium chloride-based RNA isolation protocol developed in this study demonstrated a refined and effective approach for extracting high-quality RNA from

polysaccharide-rich tissues, specifically glycogen-rich chicken liver. By incorporating modified component concentrations and additional purification steps, the protocol consistently produced RNA isolates with high purity ($A_{260}/A_{280} > 2.0$) and significantly higher yields (average 150.449 ng/ μ L) compared with both the conventional organic method and the spin-column protocol. Statistical analysis ($p = 0.004$) indicated that, while the results were comparable to those obtained using widely established methods, the optimized protocol generated significantly greater RNA concentrations. These findings suggest that the protocol has potential for application to other biological sample types; however, additional validation across diverse tissues and experimental conditions is required before broader applicability can be established. Downstream validation through cDNA synthesis and PCR amplification of the β -actin gene further demonstrated that the RNA isolates obtained using the optimized protocol were suitable for subsequent molecular applications.

Cost analysis further demonstrated that, although the lithium chloride-based method did not have the lowest per-reaction cost, its yield-to-cost ratio was higher to those of the conventional and spin-column approaches, indicating greater cost efficiency. A limitation of the protocol is its labor-intensive nature, requiring more preparation time than commercial kit-based methods.

The refined lithium chloride-based RNA isolation method offers a reliable, cost-effective, and efficient alternative for transcriptomic studies in glycogen-rich animal tissues. While the present findings support its use under the conditions evaluated in this study, further validation using a wider range of biological samples and laboratory settings is necessary to determine its broader applicability and reproducibility.

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