

ARTICLE

Efficacy of SDS For Protein Extraction from Broiler Muscles and Mammalian Liver Tissue



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Competing interests

The authors have declared that no competing interests exist.

Abstract

Background: The present study purports to check and validate the potential of sodium dodecyl sulfate (SDS) alone being a suitable and cost-effective lysis buffer for maximum and efficient protein extraction from various muscle tissues of broiler chicken and mammalian liver.

Materials and Methods: Three different muscle tissues (I; chest, II; wing and III; leg) were extracted from randomly selected commercial broilers (n=4) while mice (n=3) were dissected for the extraction of liver tissue samples. 1:1 ratio (w/v) of SDS; 10 and 1.0 & 1.5% was used for muscles and liver tissues, respectively for its best time optimization for protein extraction. After incubation, respective tissues were homogenized followed by centrifugation. The supernatant was then processed for crude protein (CP) extraction by Bromocresol Green (BCG) method.

Results: SDS (10%) achieved a maximum yield of CP after 1 hour of incubation. When checked the co-dependence of SDS-reagent on muscle-tissue type and time of incubation, tissue I (chest) was found to give maximum CP contents after 1 hour of incubation, tissue II (wing) extracted more CP after 3 hours while tissue III (leg) rendered equal amounts of CP after 1, 2 and 3 hours of incubation, respectively. From the mammalian liver tissue maximum yield of CP (6.9 g/dl), and albumin (ALB) (1.6 g/dl) was obtained with 1.5% of SDS. While the CP and albumin (Alb) content was not detected after homogenization with 1.0% SDS. Significance was checked at ($P < 0.05$).

Conclusion: It is concluded from the above findings that 10% SDS is the best lysis buffer concentration to extract crude protein from all the studied broiler muscle tissues while from mice liver samples we found 1.5% SDS lysis reagent seems good than 1.0%. Furthermore, this simple and cheapest procedure and ease of preparation this reagent may be suitable for extraction of important tissue protein fractions.

Key words: Broilers, Crude Protein, Mammalian Liver, Protein extraction, SDS

Introduction

The insights into molecular mechanisms of cell require efficient and quality procedures for yielding high cell-extracts particularly protein, lipid, nucleic acids, and other components. Protein, being an integral part of important physiological and cellular mechanisms plays a crucial role in the overall maintenance of cell growth and stability (Le Mairthee et al., 2000). Therefore, an applicable protein-extraction method is required, to generate high-yield and stable extracts from virtually all types of tissues, respectively.

The extraction procedure is a stepwise operation of mechanical and chemical nature, which starts generally with cell lyses (cell disruption). The most important step of protein extraction is to extract sufficient amount of protein with fewest contaminants, because during extraction many processes occur that affect quality of protein, such as protein unfolding, protein aggregation, degradation, and loss of function. It is best to keep protein cold during this process preferably at 4°C, to minimize proteolysis (Matsuo et al., 2006).

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There are mainly two types of extraction methods: mechanical and chemical methods. Chemical methods are further categorized, depending upon the type of ingredients. The mechanical procedures (sonication, solid agitation, freeze/thaw, use of a blender, etc.) usually produce heat and foaming, resulting in denaturation and reproducibility of protein (Mahalanabis et al., 2009; Tan & Yiap, 2009). However, these mechanical procedures are often accrued by the addition of detergents which shows efficient disruption of cellular and sub-cellular membranes. The choice of each detergent in respective procedures is highly dependent on the type of cell and extraction scale (Dhabaria et al., 2015).

The membrane proteins are isolated, purified, and crystalized with the help of detergents which act as solubilizing agents (Prive, 2007). At lower and non-solubilizing concentrations, these agents provide useful purposes as these compounds can improve the permeability of cellular membranes. These agents break various interactions i.e., protein-lipid interactions, and protein-protein interactions, and help to denature protein structures and protein crystallization. Before using detergent, it is important to have detailed knowledge about of how and in what proportion they interact with the membrane proteins and lipids (Garavito & Ferguson-Miller, 2001).

Despite the reasonable working of the detergents in improving protein stability, they are slightly effectual than charged detergents, preferably sodium dodecyl sulfate (SDS) (Dhabaria et al., 2015). Many researchers have reported the suitability of SDS buffer in various concentrations to facilitate protein extractions. In one study, Zhang et al., (2016) applied ultrasonication and 4% SDS/Urea-based lysis buffer for protein extraction and identification of major protein groups in the gut microbiome of mice and humans. While another study of Zhang et al., (2018) showed better performance of 4% (w/v) SDS protein-extraction buffer in human gut metaproteomics compared to urea and non-ionic detergent-based B-Per buffer. Previously, we have also reported a 1.0% SDS (w/v) buffer to yield maximum extractable protein (EP) from hepatic tissue of mice compared to other lysis buffers (PBS, NaOH, 0.7% SDS) included in the study, where 0.7% SDS buffer also showed a considerable yield of extractable protein respectively (Abbasi et al., 2016).

In the current study, for the first time, the concentration of SDS buffer is optimized with respect to incubation time and tissue-specificity so that one can use a reliable cost-effective, and efficient ingredient to extract protein from various broiler muscles and mammalian liver tissue.

Materials and Methods

Materials

Analytical grade SDS obtained from Sigma-Aldrich Chemie (Munich, Germany) was used. The total protein extraction kit was from Randox Laboratories, Ltd (UK).

Tissue(s) processing for protein extraction

Three different concentrations of SDS (10%, 1.5%, and 1.0%) were prepared for crude protein (CP) extraction from broiler muscles and for mice liver tissue, respectively. Briefly, whole broiler chickens were randomly obtained from a commercial hatchery (n=4). All birds were weighed individually before and after slaughtering. The procedure was performed under aseptic conditions. Edible parts: chest (Tissue I), wings (Tissue II), and legs (Tissue III) were excised, deboned immediately after slaughtering, and then washed with physiological 0.89% sodium saline. Similarly, for the isolation of protein fraction from mice liver, the animals were sacrificed followed by extraction of the liver for further processing of protein extraction for their maximum yield.

For each muscle tissue, 1:1 (w/v), 10% SDS buffer was used for homogenization separately for a brief period till further separation into their aliquots with a respective designated time of incubation. Briefly, 3 aliquots were made for each time of incubation i.e., 1, 2 & 3 hours while for liver tissue 1.5% SDS reagent was employed with 15 minutes of incubation. After incubation, all the respective tissues were homogenized at 1100 rpm for 15 min followed by centrifugation at 13200 rpm for 7 min. The supernatant obtained was then processed for estimation of total crude protein (CP) and albumin (Alb) estimation.

Statistical analysis

The data were analyzed using Prism Graph pad 8 software (San Diego, CA). Statistical significance was calculated using one-way analysis of variance (ANOVA) and Tukey post-test. Significance was accepted at $P < 0.05$ while results were shown as Mean \pm S.E.M. with n=4.

Results

The experiment interpreted the results on the basis of total protein released from each type of tissue. Using 10% SDS lysis buffer, the overall protein yield was found to be maximum in tissue I, compared to tissue II and III. Among all the sets of incubation time (i.e., 1, 2, and 3 hours), the highest protein output was given by tissue I (8.00g/dl) after one hour of incubation, while others i.e., tissue II (6.96g/dl) and tissue III (5.90g/dl) shown less yield after one hour of incubation as well as for other given sets, respectively (Table 1).

Table 1: Extracted Total Crude Protein contents of broiler chicken muscles (g/dl) after respective times of incubation in hours (H) in 10% SDS lysis reagent

Tissue Type	Incubation Time (hour)	Total protein Mean \pm S.E.M	P values
I (Chest)	1	8.000 \pm 0.889	0.215
	2	6.333 \pm 0.318	
	3	6.600 \pm 0.557	
II (Wing)	1	6.967 \pm 1.027	0.0536
	2	5.167 \pm 0.033	
	3	8.167 \pm 0.570	
III (Leg)	1	5.900 \pm 0.351	0.894
	2	6.033 \pm 0.328	
	3	6.100 \pm 0.208	

Total protein values are representatives of quadruplicate.

Tissue I (chest) shown maximum protein release after one hour incubation (8g/dl), while less extraction was obtained, even after double and three times more incubation time i.e., 6.3g/dl and 6.6g/dl, respectively. Although, a positive difference has been noted after 2 and 3 hours, it is quite insignificant, p value=0.21. Tissue II shown less extraction after one hour (6.9g/dl) two-hour incubation (5.16g/dl), but relatively high yield after three-hour incubation (8.1g/dl; $p=0.056$). However, tissue III gave a non-significant yield ($p=0.894$) after all three sets of incubation time; (1hour; 5.9g/dl, 2hour; 6g/dl, 3hour; 6.1g/dl) (Figure 1).

The comparative analysis of released total protein shown by all three tissue types suggested the existence of relatively negative co-relation with respect to each incubation time set. While taken zero as standard for comparison, results shown highest protein yield for tissue I, 13% less for tissue II, while 26% less for tissue III after 1 hour of incubation ($p=0.263$). After two-hour incubation, tissue II and tissue III extracted 18% and 4% lesser protein compared to tissue I ($p=0.048$). However, the percentage for total protein shown by tissue II was highest, compared to negative percentages of tissue I (19%) and tissue III (25%) after 3 hours of incubation ($p=0.50$), respectively (Figure 2). These findings suggest a potential relation between total protein extraction and time of incubation for each tissue type.

For the mammalian liver tissue, 1.5% SDS lysis buffer gives total protein of 6.9 g/dl. The amount of albumin by this buffer counted 1.6 g/dl. SDS 1.0% with 1:1 w/v of tissue lysis reagent does not detect the proteins

Discussion

This study is probably an extension to previously published work which preferred 0.1% SDS buffer as the optimum lysis agent over other lysis buffers (PBS, NaOH, 0.7% SDS) included in the study and hence proven to isolate more protein from hepatic tissue of mammals. However, the theme of the present study was to optimize the concentration of SDS buffer to extract crude protein (CP) with respect to incubation time and tissue-specificity from various tissues of broiler and protein fraction from liver tissue of mice. Therefore, the selection of a good type of buffer and specific technique is very important for the extraction of protein from cells. Efficient lysis and homogenization of tissues is also very important to yield a sufficient quantity of proteins (Zuidhof et al., 2014).

Results of the present study suggested that the chemical lysis with 1.5% & 10% SDS detergent alone worked well in rupturing the cells and extracting proteins immediately from mammalian liver tissue & broiler muscle tissues, respectively. This may be due to the fact that being a strong ionic detergent SDS can extract and quantitatively solubilize a large number of proteins (Hong et al., 2004). Further, on the same lysis buffer, different tissues respond differently as seen in broiler muscles and mammalian liver tissue. It might be due to the fact that some proteins readily bind with SDS, and some took time to bind, but most of the proteins were extracted after 3 hours of incubation. SDS is expensive than all other chemicals that have been used to extract proteins, but very little quantity of this chemical is used to extract proteins from large numbers of samples. This may help to stabilize cost-effectiveness of work.

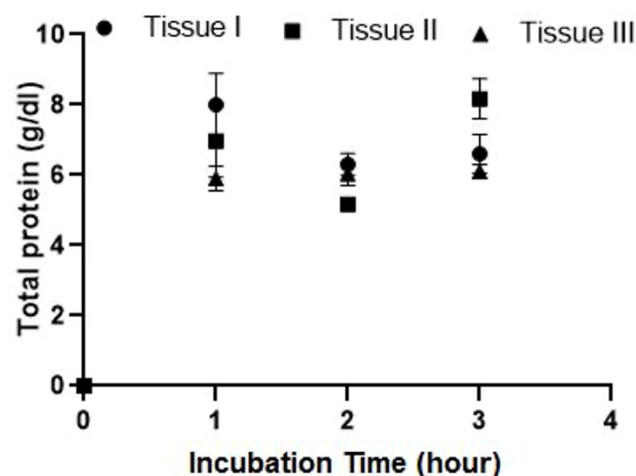


Figure 1: Extracted total crude protein contents (g/dl) from broiler muscles tissues after respective times of incubation (hour) in 10% SDS lysis

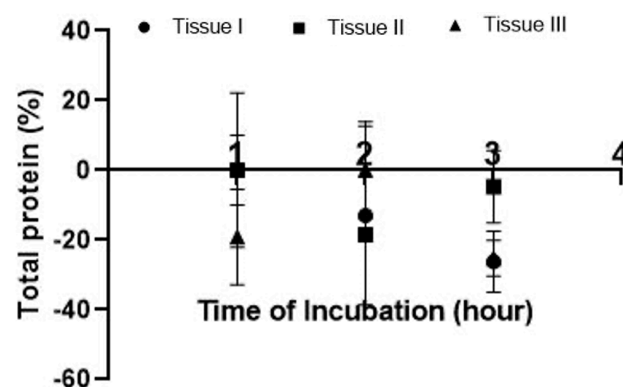


Figure 2: Extracted total crude protein contents (%) after respective times of incubation (hour) in 10% SDS lysis; zero is taken to standardize the values

Conclusion

It is concluded from the above findings that 1.5% and 10% SDS were deemed the best lysis reagent concentration to extract total protein from broilers and mice liver tissues, respectively. Thus, this lysis reagent concentration offers routine and cheaper protocols, for routine laboratory protein analysis from muscles like broiler chicken and mammalian tissue.

Author contributions

The corresponding author A Majid conceptualized the main idea of the work along with data analysis, final evaluation and drafting of the manuscript. All the co-authors conducted the laboratory work and synthesized the initial draft.

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