Gel Electrophoresis Ideally Identifies and Authenticates Cave and House Edible Bird's Nests from Common Adulterants

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ABSTRACT: Edible bird's nest (EBN) is a high price animal bioproduct found in the Southeast Asian region of the world. Primarily, EBN is used as a food in addition to its uses in several nutraceutical and pharmaceutical products. Extra care is always needed for ensuring its purity and quality. For earning extra profits, people adulterate different fake materials into EBN to raise its weight and earn extra money. This is a serious offense, and therefore, it is very important to develop methods with ability to differentiate EBN from the adulterants for the authentication of pure EBN. In this direction, we developed a gel electrophoretic method for differentiating cave and house EBNs from white fungus, jelly, fish swimming bladder and egg white. In order to identify the adulterants and authenticate EBN; efforts were made to investigate and compare the protein profiles of cave and house EBNs with white fungus, jelly, fish swimming bladder and egg white. The protein profiling indicated 10 bands for cave nests with two strong bands at 30 and 35 kDa. House nest proteins consisted of 9 bands with major bands at 120 and 140 kDa. White fungus displayed three dim bands at 22, 35 and 75 kDa whereas egg white was found to contain two predominant bands at 35 and 75 kDa. Fish swimming bladder showed substantial streaking of protein bands after dilution whereas protein profile of jelly was without any band. The results of this study can be used for the identification of any of these fake materials in EBN, and establishing the authenticity of the genuine EBN. The method reported is simple and can be used in EBN industry in the Southeast Asian region for checking the adulteration of EBN.

1. INTRODUCTION

Edible bird's nest (EBN) is a highly prized salivary bioproduct used as a health supplement in Traditional Chinese Medicine (TCM) from times immemorial. The esteem of EBN as a food with medicinal properties, and the requirement of highly skilled labourers for its collection and processing, makes it an expensive bioproduct. Presently, there are much increasing demands of the cave EBNs despite of their low production level (Marcone, 2005). As a consequence of these facts, various fake materials such as Tremella fungus, karaya gum, pork skin, jelly, fish swimming bladder and egg white are being adulterated into EBN for increasing weight before sale for higher profits (Wu, et al., 2010). Normally, the adulterants are quite difficult to identify without serious examination because of similar colour, physical appearance, taste and texture to that of the salivary nest cement. Adulterated or fake EBN may be quite dangerous to the consumers. Therefore, the identification of fake materials and ensuring the authenticity and the quality of EBN is very important.

Protein electrophoresis is used to analyze proteins in a liquid sample or an extract of the analyte. Generally, electrophoresis can be carried out with small sample concentrations in several alternative ways with or without the use of a supporting medium (Liu, et al., 2012). Electrophoretic techniques are powerful, cost effective, simple and easy for use in the fractionation of complex protein mixtures, valuation of sample concentration and complexity, and the removal of interfering contaminants. To the best of our knowledge, this is the debut approach utilizing the efforts of gel electrophoresis for the identification of some common adulterants in EBN.

2. EXPERIMENT

2.1 Sample Collection

Cave and house nests were obtained from Gua Niah (Niah National Park), Sarawak, Malaysia and swiftlet premises (professional suppliers) in Batu Pahat, Malaysia, respectively. Dried chips of tremella fungus, jelly and fish swimming bladder were procured from a grocery store in Skudai, Malaysia. Pure egg white albumin was supplied by Promga Corporation, Madison, USA.

2.2 Chemicals, Reagents and Apparatus

All the chemicals and reagents were of analytical reagent grade and used without further purification.

Electrophoresis buffer, acrylamide (electrophoresis grade), bis-acrylamide (N,N´-methylenebisacrylamide), tris (2-hydroxymethyl-2-methyl-1,3-propanediol), SDS (sodium dodecyl sulphate or lauryl sulphate), TEMED (N,N,N´,N´-tetramethylenediamine-ethylenediamine), ammonium persulphate, 2-mercaptoethanol, glycerol, bromophenol blue, glycine, Coomassie Blue R-250 and Precision Plus Protein Dual Colour Standards were purchased from Bio Rad Laboratories, Hercules, USA. Minigel apparatus (Bio-Rad Mini-Protean III apparatus) connected to a power supply (capacity 200 V, 500 mA) was used for gel electrophoresis.

2.3 Preparation of Crude Protein Extracts from Raw Samples

The raw samples of cave and house nests were cleaned manually by removing dirt, feathers and egg shells. The cleaned samples (cave and house nests, white fungus, jelly and fish swimming bladder) were separately ground in a mortar and screened through a 1 mm steel filter. Crude egg white sample was prepared by dissolving 0.01 g pure egg white albumin with 20 ml of distilled water. Crude proteins from the raw samples were extracted by aqueous extraction. Alkaline extraction and water extraction methods were applied for the extraction of proteins from the as-prepared samples.

2.3.1 Alkaline Extraction

1 g of each raw sample was immersed in 30 ml of 0.25 N NaOH solution for 48 h. This was repeated for NaOH solutions with molarities 0.1 and 0.4 M. Then, the aliquot of each extract was immersed in water bath at 65 ºC for 2 h. The extracted solutions were centrifuged and eventually the supernatant was obtained.
After the extraction processes, the suspensions were centrifuged at 18000 rpm for 20 minutes, and the supernatants were dialysed thoroughly against distilled water.

2.3.2 Water Extraction

This extraction method was adapted from Goh et al. (Goh, et al., 2001), Oda et al. (Oda, et al., 1998) and Kong et al. (Kong, et al., 1987) with minor modifications. 1 g of each raw sample was suspended in 30 ml deionized water and allowed to elute for 48 h at 4 ºC. Then, the aliquot of each extract was immersed in water bath at 65 ºC for 2 h. The extracted solutions were centrifuged and eventually the supernatant was obtained. The extraction process was repeated with changing the temperature of decoction to 75, 85 and 95 ºC.

2.4 Protein Profiling

Protein profiling was carried out by gel electrophoresis using the method of Bollag et al. (Bollag, et al., 1996).

2.4.1 Gel Electrophoresis

Acrylamide gel electrophoresis was used to separate proteins on account of the differences in their molecular weights. For the determination of the acrylamide percentage, the range of molecular weight was forecasted. For the present study, the range of molecular weight of bird's nest protein was approximately 14-97 kDa (Goh, et al., 2001). The fitting acrylamide percentage in separating gel was 12.5%.

3. RESULTS AND DISCUSSION

Protein constituents of foods and other materials are different from each other and therefore, every such item should have different protein profiles. This was the basis for the analysis of protein profiles as a means of the identification and authentication of EBN. Basically, protein analysis involves two main steps, viz. sample extraction and gel electrophoretic analysis. Sample preparation is crucial to the clear and accurate resolution of protein bands (Burgess, 2008). For that reason, the raw materials were ground and sieved so that samples with large surface area were obtained. During the extraction process, the soluble proteins were dissolved leaving behind the insoluble proteins. However, this procedure was excluded for the egg white sample because it was purchased in pure form.

3.1 Protein Profiles

A series of extraction processes was tried out to find out the most suitable results. Both alkaline and water extraction strategies were applied for protein extraction. Their effectiveness for extraction was compared, and the best method was selected to process the samples under investigation for ensuring a standardization for all the samples.
0.1, 0.25 and 0.4 M NaOH concentrations were used for the alkaline extraction processes. Different patterns of protein bands were obtained in all the samples. So, it was obvious that alkaline extraction is futile for protein profiling of the samples. Water extraction with temperature gradient analysis was also carried out to identify the best temperature for improving the protein extraction and reducing the possibility of protein hydrolysis. The water extraction experiments were carried out at 65, 75, 85 and 95 °C. Overall, the experiments indicated that the number of polypeptides and the molecular weight of the protein fractions varied between alkaline and water extraction methods. Water extraction was chosen as the standardized method to extract the proteins of all samples under investigation due to significant protein profiles obtained with less smearing and good resolution of bands. From the temperature gradient analysis, 75 °C was established as the best temperature for water extraction process because the protein profile of the samples produced by water extraction at this temperature showed more substantial

bands except for fish swimming bladder and jelly.

After protein extraction, the second step of sample preparation is to adjust the sample concentration by dilution so that an appropriate amount of protein is loaded into the gel. Different dilution factors were used to dilute each of the concentrated samples. By comparing the protein profile produced for each dilution (**Figures 1-5**), the most suitable dilution factor was determined for each sample. Each sample possessed its own unique protein profile (except jelly). Besides, the protein profile of each sample except fish swimming bladder and jelly exhibited pronounced bands after dilution. On increasing the dilution gradually, the bands become faint and finally disappeared, and as a result the best dilution conditions were achieved. On the basis of the suitable dilutions, the protein profile of each sample was obtained for comparison.

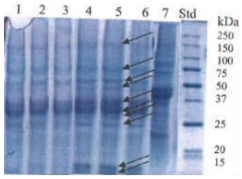

Figure 1: Protein profile of cave nest sample produced after dilution. Lane 1 (50:50), Lane 2 (60:30), Lane 3 (70:30), Lane 4 (80:20), Lane 5 (90:10), Lane 6 (95:5) and Lane 7 (No dilution).

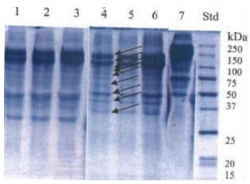

Figure 2: Protein profile of house nest sample produced after dilution. Lane 1 (30:70), Lane 2 (40:60), Lane 3 (50:50), Lane 4 (60:40), Lane 5 (70:30), Lane 6 (80:20) and Lane 7 (No dilution).

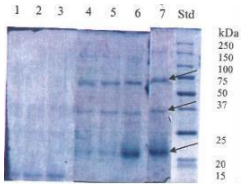

Figure 3: Protein profile of white fungus sample produced after dilution. Lane 1 (40:60), Lane 2 (50:50), Lane 3 (60:40), Lane 4 (70:30), Lane 5 (80:20), Lane 6 (90:10) and Lane 7 (No dilution).

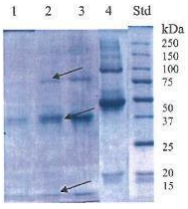

Figure 4: Protein profile of egg white sample produced after dilution. Lane 1 (50:50), Lane 2 (60:40), Lane 3 (70:30) and Lane 4 (No dilution).

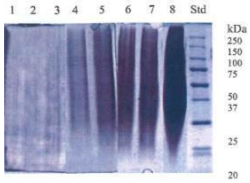

Figure 5: Protein profile of fish swimming bladder sample produced after dilution. Lane 1 (5:120), Lane 2 (5:110), Lane 3 (5:100), Lane 4 (5:95), Lane 5 (10:90), Lane 6 (20:80) Lane 7 (30:70) and Lane 8 (No dilution).

Figure 1 shows that the protein bands of cave nest consisted of 10 bands with the strong bands at 30 and 35 kDa. Six more medium bands located at 12, 25, 28, 42, 50 and 60 kDa were also found; indicating that the cave nest protein contained various types of polypeptide chains at different proportions.

Figure 2 shows that the protein profile of house nest consisted of nine bands, and two of the bands were identified at 120 and 140 kDa, which were absent in the protein profile of cave nest. The protein profile additionally displayed seven medium bands at 35, 45, 50, 60, 70, 80 and 100 kDa. **Figures 3** and **4** show some common bands in both nest types at 50 and 60 kDa. Thus, it may be concluded that the cave and house nests consisted of different protein compositions though they have been produced by the same swiflet species (Collocalia).

Figure 3 shows three dim bands at 22, 35 and 75 kDa for white fungus sample. The 35 kDa polypeptide of the white fungus is similar to one of the protein bands of cave nest. But the protein fraction of cave nest has been slightly stronger in comparison to the white fungus sample. Three bands were detected in the egg white sample, which were absent in other samples (**Figure 4**).

Figure 5 shows substantial streaking in the protein profile of fish swimming bladder. However, a band was detected after dilution around 31 kDa.

Thus, a clear picture of the protein profiles of cave and house EBNs, white fungus, fish swimming bladder, egg white and jelly were obtained. A comparison of the protein profiles of both the types of EBNs with these materials (common adulterants) can be used for distinguishing between EBN and these materials.

4. CONCLUSION

Gel electrophoretic method was used for protein profiling studies of cave and house nests, white fungus, fish swimming bladder, jelly and egg white. Water extraction at 75 °C was chosen for protein profiling studies of the

crude samples. Protein profiling of cave nest revealed 10 bands with two strong bands at 30 and 35 kDa. On the other hand, there were 9 bands with major bands at 120 and 140 kDa in the protein profile of house nest. Three dim bands at 22, 35 and 75 kDa were obtained in white fungus, whereas egg white was found to contain two predominant bands at 35 and 75 kDa. Substantial streaking was obtained in the protein profile of fish swimming bladder after dilution. The protein profile of jelly was without any band. Overall, each sample had a unique protein profile except jelly. Thus, a clear picture of the actual protein profiles of EBN and some commonly used adulterants has been presented. SDS PAGE adequately differentiated EBN from other adulterants. The results of this analysis can be used for the identification and authentication of any of these fake materials in EBN.

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