# CHARACTERIZATION AND STANDARDIZATION OF EDIBLE BIRDS NEST (EBN)-DETERMINATION OF SIALIC ACID

Thavamanithevi, S.<sup>1\*</sup>, R. Sarifah<sup>1</sup>, C.G. Lim<sup>1</sup>, M. Theanmalar<sup>1</sup>, M.S. Aidawati<sup>1</sup>, M. Durgah Devi<sup>1</sup> and A.A. Saleha<sup>2</sup>

<sup>1</sup>IBRC B19, SIRIM Berhad, No 1 Persiaran Dato Menteri, Section 2, P.O. Box 7035 40700 Shah Alam, Selangor, Malaysia. <sup>2</sup>Jabatan Veterinar Universiti Putra Malaysia \*thava@sirim.my

#### Introduction

Due to the rise in the rate of adulterated edible bird nest (EBN), effective analytical methods were explored to detect the authenticity of edible bird nest <sup>[1]</sup>. The data collected could be used as a guide to establish standardized EBN against sialic acid content of the nest that will safeguard the quality of local edible bird nest produced for export and consumption. This study was aimed to develop a standardized method in detecting and quantifying sialic acid found in EBN. Current chromatographic methods that have been reported use a large sample size which is hydrolysed and derivatives were analysed in the HPLC system using PDA detector. From our study we have established a method to quantify sialic acid without derivatization and using a minimum quantity of dried EBN sample (50mg).

The aim of this study was to determine the authenticity of edible bird nest through the analysis of sialic acid content. The natural variation due to geographical location and seasonal variation was also statistically evaluated.

#### **Materials and Method**

The Raw-clean EBN samples were dried in the oven overnight at  $60^{\circ}$ C and then grounded to powder. A sample of 50mg of the powdered were dissolved and hydrolysed in 2.5ml acetic acid (1%) solution. Sample was vortex to ensure complete mixing of the samples during hydrolysis. The sample was then placed in a water bath at  $80^{\circ}$ C for 30 minutes, later cooled and 2.5 ml of phosphoric acid solution (1%) is then added into the sample solution and made up to mark using 5 mL volumetric flask.

Quantification of sialic acid using HPLC method; the extract was subjected to HPLC analysis with photodiode array detector using gradient elution technique (water: phosphoric acid) for detection of sialic acid.

#### **Result and Discussion**

The EBN samples were sourced from different states according to the northern, middle and eastern region. Most of the nests received were purchased from farmers and some were given as free samples. The samples were also mostly from the house nest and a few from the cave nest (Sarawak). Even though the nest originated from the same region, there were variations in the colour of the samples. The cave nest from Sarawak varied from Off white yellowish, Yellow and red coloured. These could be due to the environment of the cave dwellings and the difference in the minerals.

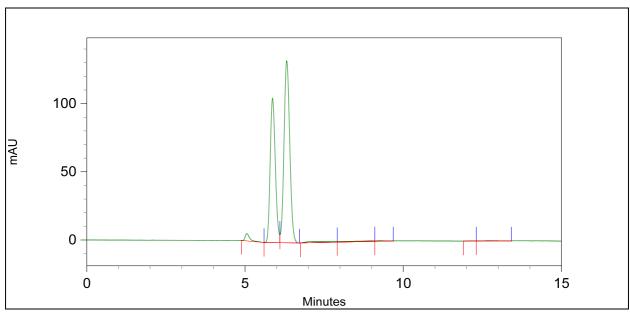


Figure 1: HPLC Chromatogram of N-Acetylneuraminic acid and N-Glyconeuraminic Acid with retention time 6.31minutes and 5.87 minutes respectively.

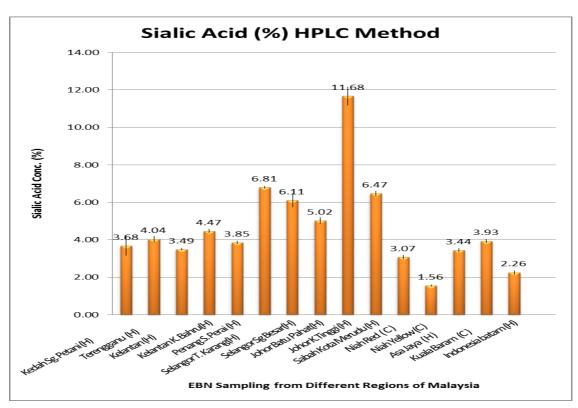


Figure 2 : Sialic Acid in EBN samples: \*(H) denotes house nest and (C) : Cave nest

From the quantification of sialic acid: the northern regions' (Kedah, Terengganu, Kelantan and Penang) sialic acid content did not vary much in the range of 3.5 to 4.5 %. The central region from Selangor had a higher content of about 6 %, while the Southern region mainly from Johor had a variation of 6 - 11%. It may be due to the sampling method. The eastern region which comprise of the house and the cave nest had a variation of 1 to 6 %. Overall the difference of sialic acid content did not vary much.

#### Conclusion

Various analytical methods were used to quantify the sialic acid content of the EBN using chromatographic analysis such as sialic acid kit, HPLC and Fluorescence method. The data collected could be used as a guide to establish standardised EBN against sialic acid content of the nest that will safeguard the quality of local edible bird nest produced for export and consumption. From the various study that was conducted the HPLC method without the need to derivative the sample was the most standardized method.

This method could be recommended to be used for the standard method for sialic acid quantification. The sample solution is then analysed using HPLC without derivatizing the sample. The sample used for quantification was minimal in the range of 50mg. With this project we manage to reduce the amount of EBN sample. The method for quantification of sialic acid was done without the need to derivative the sample using HPLC and PDA method.

## Acknowledgement

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### References

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