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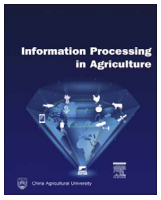

Preliminary nitrite, nitrate and colour analysis of Malaysian edible bird's nest

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ABSTRACT

The high nitrite content in edible bird's nests is a major concern to the local swiftlet industry. It lowers the price of the edible bird's nests and it brings severe health hazards to consumers and farmers. This research investigated the nitrite and nitrate contents of eight types of local edible bird's nests by using ion chromatography system and evaluating its colour using the CIE system in $L^*a^*b^*$ parameters. The nitrite content obtained ranged from 5.7 μg/g for the house nests to 843.8 μg/g for the cave nests. The nitrate content for the house and cave nests was 98.2 μg/g and 36,999.4 μg/g, respectively. The cave nests with darker and redder colour had higher nitrite and nitrate contents than the brighter and more yellow house nests. This likely suggests that the nitrite and nitrate contents have correlations with edible bird's nests colour. Correlations studies suggested that the nitrite content had high correlations with colour parameters, $L^*a^*b^*$ of edible bird's nests at significant level of $P < 0.10$. These findings suggest that edible bird's nests' colour may be a useful indicator for measuring nitrite and nitrate contaminations.

1. Introduction

Edible bird's nest is also known as *Yan Wo*. It is one of the most expensive animal products of saliva secretion produced from two specific swiftlets, namely the *Aerodramus fuciphagus* and *Aerodramus maximus* [1]. The white-nests produced by *A. fuciphagus* are usually marketed at a higher price compared to the black-nests from the *A. maximus*. This is due to the consumers' perceptions that white-nests have higher nutritional values. Edible bird's nest contains high nutritional content such as protein, carbohydrates, mineral salts and amino acids [2–4]. It also possess high medicinal benefits in enhancing complexion, strengthening immune system, stimulating epidermal growth, depressing the production of tumour necrosis factor-alpha, inhibiting influenza virus infection and improving respiratory and digestive problems [5–9]. Edible bird's nest is widely consumed and used in the Chinese commodities, especially in China and Hong Kong. There was an estimated market of the edible bird's nests of about HK$3 billions since 2004 in Hong Kong [10]. The edible bird's nest is commonly found in derivatives such as soups,

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beverages, cuisines as well as cosmetics and skin care products. The colour of edible bird's nest usually range from white and ivory for house nests to yellow and brown for cave nests. Colour is an important attribute which can serve as an indicator for food quality and also closely related to consumer food acceptability [11].

The nitrite and nitrate are inorganic compounds that naturally present in the environment such as water and soil. Nitrite is commonly used in food industry as food preservative to prevent spoilage and to maintain the colour of meat and meat products such as bacon, ham, luncheon, corned beef, hamburger and smoked fish [12]. However, there is a concern of association of nitrite contents with cancers. The nitrite can be harmful to human health when it reacts with secondary amines and amides in the gastrointestinal tract to form carcinogenic *N*-nitrosamines which can cause stomach cancer [13–15]. Reports from the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2002 suggested that the acceptable human daily intake amount of nitrite should not exceed 0.07 mg/kg of body weight. The major source of nitrate in food comes from water and many vegetables such as spinach, eggplants, lettuce, radishes and beets because it is a natural constituent in plants [12]. High intake of nitrate can be hazardous to health because nitrate is a potential source of nitrite through bacterial or microbial reduction [12,13]. Nitrite is more toxic than nitrate because nitrite interacts with blood pigment to produce methemoglobinemia which caused blood disorder and breathing difficulty in human [12,13].

There is an increase in public concern on the high level of nitrite in Malaysian edible bird's nests after incidences of the edible bird's nests products being banned during export [16]. The nitrite and nitrate contaminations in edible bird's nests were contributed by the fermentation process of bird soil and guano [16] and the natural environment resources such as atmosphere, water, and soil [17]. The nitrite level in edible bird's nests should be controlled strictly to ensure its safety and quality for consumption. This research aims to measure the nitrite and nitrate contents of Malaysian edible bird's nests obtained from farm houses and caves. The edible bird's nests from farm houses are those from the swiftlet rearing activities where the swiftlets are attracted to build its nest in cave-imitating structures in man-made buildings using swiftlets' recording voice. The edible bird's nests from caves are from natural nesting activities of the swiftlets. The colour of edible bird's nest was also measured to evaluate its correlations with the nitrite and nitrate contents.

2. Materials and methods

2.1. Edible bird's nest powder preparation

A total of eight unprocessed Malaysian edible bird's nests were collected in Malaysia, comprising of four house nests (H) and four cave nests (C). The house nests were labelled as H1 (Segamat, Johor), H2 (Kapar, Selangor), H3 (Nibong Tebal, Penang) and H4 (Sarikei, Sarawak), and cave nests as C1 (Gua Gomantong, Sabah), C2 and C3 (Gua Niah, Sarawak) and C4 (Gua Subis, Sarawak). The edible bird's nests were refrigerated at 4 °C before grinded into powder using a high speed dry grinder (MFM-202, Ta Feng Electrical Appliances Co. Ltd., Taiwan) for 30 s. The powder was sieved using analytical sieve shaker (AS 200, Retsch, Germany) with 1 mm mesh size. The powder was kept in an airtight plastic container at 4 °C prior to experiments.

2.2. Nitrite and nitrate determination

The nitrite and nitrate contents of edible bird's nests were determined using the ion chromatography system. This method was based on Malaysian Standard MS 2509:2012 (P), Dionex application note 133 and Dionex application update 131 [16]. The edible bird's nest extracts were prepared by mixing of 0.5 g of powder with 20 ml of ultrapure water (1:40 w/v). The mixture was immersed in a water bath at 80 °C for 30 min, followed by centrifugation with a centrifuge (accuSpin™ 1, Fisher Scientific, Germany) at 3500 rpm for 15 min. The supernatant was filtered through a 0.45 μm filter. A 20 μl of filtrate or extract was injected into an ion chromatography (ICS-90, Dionex Corporation, USA) for analysis. All analyses were performed in duplicates. A five point external calibration curves for seven standard anions were constructed with high linearity (R^2 = 0.999–1.0). Standard curves were used for nitrite and nitrate identification and quantification of edible bird's nests. The instrument detection limit was at 0.1 ppm. The Chromeleon 6 chromatography software was used to compare the data from edible bird's nests to standard and automatically converted each peak area in a chromatogram to concentration following Eq. (1):

$$A = \frac{C \times V \times F \times 1000}{W \times 1000} \quad (1)$$

where A = amount of nitrite or nitrate in the sample, μg/g; C = amount of nitrite or nitrate in the sample obtained from the calibrated curve, mg/l; V = volume of sample solution, ml; F = dilution factor of sample solution; W = weight of sample, g.

2.3. Colour determination

Colour parameters of edible bird's nests were determined using International Commission on Illumination (CIE) $L^*a^*b^*$ system where L^* is lightness, a^* is red/green ($+a^*/-a^*$) and b^* is yellow/blue ($+b^*/-b^*$). The colour was measured by placing whole edible bird's nest perpendicular to the optical sensor of a colour reader (CR-10, Minolta Camera Co. Ltd., Japan). Three pieces of edible bird's nests were randomly selected from each nest types, and ten readings were taken from each piece of edible bird's nest [2]. This analysis was performed in duplicates.

2.4. Statistical analysis

The Analysis of variance (ANOVA) and correlation analysis were conducted using Minitab software (Version 16, Minitab Inc., USA). One-way ANOVA followed by Tukey's test was carried out for the nitrite and nitrate contents, and also the colour parameters of edible bird's nests. Significant differences among means were defined at 95% confidence levels ($P < 0.05$) and demonstrated in different letters as shown in the graphs. Correlations were established using Pearson's