PRELIMINARY ASSESSMENT OF ALUMINIUM AND CITRIC ACID CONTENT IN RAW-UNCLEAN (RUC) AND RAW-CLEAN (RC) EDIBLE BIRD'S NEST (EBN) IN MALAYSIA

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ABSTRACT. Edible bird's nest (EBN) are highly prized health food delicacies, particularly among Chinese communities worldwide, with Malaysia being a key exporter to China. This study aimed to determine initial reference levels of aluminium and citric acid in EBN from Malaysia, responding to concerns raised by China Customs about these components. A total of 32 EBN samples, comprising of raw-unclean (RUC) and raw-clean (RC) varieties, were analysed using inductively coupled plasma optical emission spectrometry (ICP-OES) for aluminium and high-performance liquid chromatography (HPLC) for citric acid. Results showed significant differences in aluminium and citric acid concentrations between RUC and RC samples. For combined EBN samples, the reference range for aluminium content can reach up to 25.39 mg/kg, while citric acid extended to 4979.23 mg/kg. In RUC samples, aluminium levels peaked at 33.22 mg/kg and citric acid at 6688.65 mg/kg, whereas RC samples showed lower values, with aluminium up to 20.82 mg/kg and citric acid up to 3341.93 mg/kg. Statistical analysis of 15 paired samples confirmed that cleaning process significantly reduced aluminium ($p = 0.012$) and citric acid ($p < 0.001$) contents. These findings provide an initial reference for aluminium and citric acid levels in Malaysian EBN, demonstrating the effectiveness of cleaning process in reducing potential contaminants and offering valuable data for quality control and regulatory compliance in the EBN industry.

Keywords: Edible Bird's Nest, Aluminium, Citric Acid, RUC, RC

INTRODUCTION

Edible bird's nest (EBN) is a highly prized delicacy in many parts of Asia, known for their nutritional benefits. Malaysia is one of the leading producers of EBN, contributing significantly to both domestic consumption and export markets. However, concerns regarding the contamination of EBN with harmful substances like heavy metals and excessive processing chemicals have raised food safety issues in recent years. The issues of aluminium and citric acid content in EBN were highlighted by China Customs during the 2024 Guangdong Bird's Nest Industry Association Annual Conference, themed "Smart Innovation, Steady Progress." The event was held at the Guangzhou International Health Ark on March 13-14, 2024. Concerns were raised by the Xiamen Customs Technical Center, through presentation on customs safety regulations, regarding the continuous detection of high concentrations of these components in imported raw-clean (RC) EBN from Indonesia and Malaysia. Limited literature is available to date regarding these two components in EBN.

Aluminium, the third most abundant element in the Earth's crust (8.2% by mass), is widely present in the environment. It occurs as compounds like silicates, oxides, and sulfates due to its high reactivity (Abubakar, 2020). Its commercial production began in 1856 by Sainte-

Claire Deville, launching its use across industries (Abubakar, 2020). Aluminium enters daily life via food, water, medications, and air, stemming from natural sources, food additives, cooking utensils, and packaging (Soni *et al*., 2001; Niu, 2018; Alasfar & Isaifan, 2021). Dietary intake varies, averaging 7–9 mg/day for adults, 0.7 mg/day for infants, and up to 11.5 mg/day for adolescents, with some foods like fried dough reaching 514.6–1578.6 mg/kg (Soni *et al*., 2001; Niu, 2018). Gastrointestinal bioavailability is low (<1%), though citric acid can increase absorption, while silicon may reduce it (Soni *et al*., 2001; Niu, 2018). Concerns about aluminium's health effects include neurotoxicity, linked to occupational exposure or direct brain contact, and bone disorders like osteomalacia, especially in individuals with renal impairment or infants on parenteral nutrition (Igbokwe *et al*., 2019; Alasfar & Isaifan, 2021). Although its role in diseases like Alzheimer's remains unproven under typical dietary conditions, monitoring exposure is advised for vulnerable groups (Stahl *et al*., 2011). This background highlights the need to assess aluminium in EBN, where contamination risks are emerging and in lieu of EBN as health food given specially to elders and people with impaired health to strengthen their immune system.

To date, only a limited number of studies have examined aluminium content in EBN, revealing significant variability across different nest types and processing stages. One study analysed 61 verified EBN samples from Malaysia, Indonesia and Thailand, reporting aluminium levels ranging from 0.002788 ppm to 0.0233 ppm (analysed with ICP-MS), with cave nests generally exhibiting higher aluminium content compared to house nests (Ma *et al*., 2020). Another study focused on 17 EBN samples provided by the Ministry of Health, comprising 7 house nests and 10 cave nests. Aluminium levels ranged from 5.58 ppm to 4366.00 ppm (analysed with Neutron Activation Analysis (NAA) technique). The findings again highlighted the tendency for cave nests to have elevated aluminium levels (Salim *et al*., 2018). Furthermore, one study specifically investigated raw and commercial (processed) EBN. The results showed significantly higher aluminium levels in commercial EBN purchased from Chinese traditional medicine shops in Malaysia, with an average of 11.92 ppm (range: 0.43468 ppm to 39.05598 ppm). In contrast, raw unclean (RUC) EBN purchased from house farms in Malaysia, with an average of 0.91297 ppm (range: 0.230875 ppm to 6.43731 ppm). These findings emphasize the potential influence of environmental exposure and processing activities on aluminium contamination (Chen *et al*., 2014).

In addition to aluminium, citric acid has also been highlighted by the China Customs, where high concentrations were detected in imported EBN. Citric acid ($C_6H_8O_7$) is a weak organic acid widely used in the food industry. It is generally recognised as safe (GRAS) by the FAO/WHO, with no limit on Acceptable Daily Intake (ADI) (Książek, 2024). Naturally present in citrus fruits like lemons (up to 8% by weight), most citric acid in food is manufactured via fermentation with Aspergillus niger (Książek, 2024; Show *et al*., 2015; Sweis & Cressey, 2018). Manufactured citric acid (MCA) serves as a flavouring agent, preservative, and acidulant in processed foods and beverages, leading to frequent dietary and dermal exposure (Chen *et al*., 2014; Booth & Morgan, n.d.; Sweis & Cressey, 2018). It offers antioxidant, antimicrobial, and versatile properties, such as chelating metal ions and enhancing flavour (Książek, 2024; Show *et al*., 2015). However, concerns regarding MCA have been raised. Reported effects include potential inflammatory reactions (e.g., joint pain, gastrointestinal issues), liver damage at high doses, and cell apoptosis. These effects appear to be dose-dependent (Booth & Morgan, 2024; Sweis & Cressey, 2018; Chen *et al*., 2014). Its production using Aspergillus niger, an allergen, has prompted calls for further safety research despite its GRAS status (Sweis & Cressey, 2018).

Currently, only two studies have investigated the concentration of citric acid in EBN. Xing *et al*. (2024) analysed the relative content of citric acid in EBN using high-and low-field nuclear magnetic resonance (HF/LF-NMR), where the relative content was calculated from the integrated area of spectral peaks. Their findings identified the following citric acid relative contents (in percent) in raw-clean (RC) EBN processed in laboratories: Malaysia (0.954±0.016), Vietnam (0.648±0.017), and Indonesia (0.825±0.026) (Xing *et al*., 2024). Another study by Chan *et al*. (2015) assessed the citric acid concentration in RC EBN samples purchased from the Hong Kong market using LC-MS/MS. The study reported a citric acid concentration range of 0.00-2.04 g/kg in white EBN and 1.05-4.03 g/kg in red EBN (Chan *et al*., 2015). The study also highlighted the impact of processing on citric acid content. They reported that citric acid in EBN decreased by approximately 90% following standard cooking processes. This significant reduction the author suggested was due to citric acid primarily existing in a free salt form within EBN, which is highly susceptible to degradation during thermal processing.

Based on the combined evidence from several studies, EFSA has recommended a tolerable weekly intake (TWI) of 1 mg aluminium/kg body weight/week. In 2011, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed new scientific evidence which enable the organization to reevaluate the risk assessment of aluminium intake. This resulted in increased Provisional Tolerable Weekly Intake (PTWI) of aluminium at 2 mg/kg of body weight, as proven by the evidence submitted that showed it is safe for people to consume up to this level without appreciable risk to their health.

Although citric acid is generally recognised as safe (GRAS) by regulatory agencies such as the FDA, concentrated forms may cause skin and eye irritation. Therefore, appropriate safety measures, such as gloves and eye protection, are necessary. FAO/WHO has set limits for citric acid as a food additive in specific foods (not all food): complementary foods for infants and young children (5000 mg/kg), concentrates for fruit juices (3000 mg/kg), and concentrates for fruit nectar (5000 mg/kg). The national standard of the People's Republic of China (GB5009.157-2016) sets the limit of citric acid for specific foods, including: (A) juice/juice drink/jelly/canned fruit at 250 mg/kg, (B) gum-based candy/bread/pastry/baked food fillings at 500 mg/kg, and (C) solid beverages at 50 mg/kg.

This research addresses the detection of aluminium and citric acid in EBN, an issue raised by China Customs during a conference in Guangzhou on March 13-14, 2024. In response, Malaysia's Department of Veterinary Services (DVS) emphasized its importance, proposing collaboration between researchers and industry to evaluate initial concentrations of aluminium and citric acid in RUC and RC EBN from Malaysia. Such preliminary assessments are vital for establishing starting points before implementing food safety programs. They provide a reference for tracking changes and evaluating interventions aimed at improving quality and nutrition. This study aims to determine the concentration ranges of aluminium and citric acid in RUC and RC EBN, provide an initial reference for Malaysia, and compare levels within the same batch to evaluate changes due to cleaning. These findings seek to offer a preliminary reference for these contaminants in Malaysian EBN, supporting quality control and regulatory compliance.

MATERIALS AND METHOD

Sample Collection

A total of 32 EBN samples were collected from various regions across Malaysia, spanning Peninsular Malaysia and East Malaysia, provided by 11 companies (Figure 1). The samples originated from the states of Perak, Sabah, Sarawak, Pahang, Johor, Selangor, Kedah, and Kelantan. From the 32 samples, 29 were house nests and three were cave nests. The samples were classified into two categories: RUC and RC. RUC samples represent EBN harvested from swiftlet houses or caves with minimal processing, while RC samples underwent preliminary cleaning procedures.

Among the 32 samples, 30 (15 pairs) were prepared for comparison. Each pair consisted of EBN from the same batch, processed as follows: approximately 25-30 g of RUC EBN was collected from the same birdhouse or batch. The RUC EBN was dried overnight, and the moisture content was tested to ensure it was below 15%, using a moisture content meter (Victor, China). The RUC EBN were then weighted to achieve a target dry weight of at least 25 g. Each RUC EBN sample was split into two equal halves. One half was stored in a sealed plastic bag or container, and the other was processed to produce RC EBN. The RC EBN, with a moisture content of less than 15%, were sealed separately. Both the RUC and RC EBN samples were sent to CAIQ Biosecurity Sdn Bhd for testing. At the laboratory, the samples were homogenized (Fritsch International, Germany) prior to analysis. Two additional RUC samples, provided by two companies, were not cleaned prior to being sent for testing.

For preliminary studies, the total sample size of 32 EBN were considered adequate to capture variability across major production regions in Malaysia (Peninsular and East Malaysia) and to ensure representation of both house and cave nests. The inclusion of paired RUC and RC samples (N=30, forming 15 pairs) allowed for direct within-batch comparisons, strengthening the reliability of results while balancing feasibility, resource constraints, and industry sampling practices.

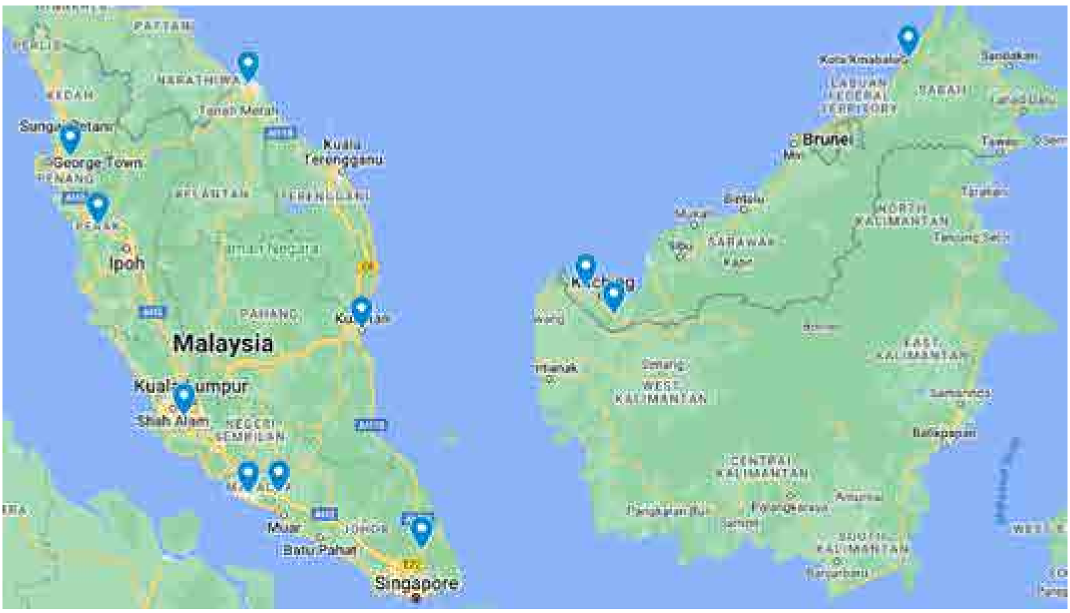

Figure 1: Geographical distribution of the 11 companies across Peninsular Malaysia and East Malaysia that supplied EBN samples for this study

Analytical Techniques

Determination of Aluminium content in EBN

The concentration of aluminium in EBN samples was determined following the procedures outlined in National Food Safety Standard - Determination of Multi-elements in Foods (2016), GB 5009.268-2016 Part II, a national food safety standard established in China for the determination of multi-elements in foods, including aluminium. Aluminium analysis was performed using inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Scientific iCAP 6300, USA) following sample digestion in microwave (Anton Paar GmbH, Austria) with concentrated 65% analytical grade nitric acid (Chemiz, Malaysia).

Approximately 1.0± 0.1 g of homogenized EBN was weighed into each microwave digestion vessel, then added with 6 mL of concentrated nitric acid. The vessels were loosely capped and left to react at room temperature for 1 hour. The vessels were then securely tightened and subjected to microwave-assisted digestion (The Anton Paar Multiwave Go, Anton Paar GmbH, Austria) under controlled conditions: temperature ramping, from room temperature to 180 °C over 20 minutes, then maintained at 180°C for 10 minutes. Upon completion of digestion, the solutions were allowed to cool to room temperature before being filtered through Whatman 41 equivalent filter paper into 25 mL volumetric flasks. The filter paper was subsequently rinsed multiple times with distilled water to ensure complete transfer of analytes. Finally, the filtrates were brought up to volume (25 mL) with distilled water before analysis. Aluminium quantification was performed at an analytical wavelength of 309.271 nm. The instrument was calibrated using a series of standard solutions with concentrations of 0.05, 0. 1, 0. 2, 0. 5, 1, and 5 mg/kg. Aluminium standard solutions (1000 mg/L, Sigma Aldrich) were used for calibration. The limit of detection (LOD) for aluminium was 0.01 mg/kg, while the limit of quantitation (LOQ) was 0.05 mg/kg.

Quality control procedures included the use of reagent blanks, duplicate samples/ digestions, and spiked recoveries to monitor contamination, reproducibility, and method accuracy. Method validation was assessed by evaluating precision (relative standard deviation (RSD) < 5% across replicates), and recovery rates, which ranged between 92% and 105%. These recovery values fall within accepted range for food safety analysis (90-110%). These results confirm the accuracy and robustness of the method despite minor matrix effects.

Determination of citric acid content in EBN

The concentration of citric acid in EBN samples was determined following the procedures outlined in National Food Safety Standard - Determination of Organic Acids in Foods (2016), GB 5009.157-2016, a national food safety standard in China that focuses on the determination of organic acids including the citric acid in food products. This standard provides detailed methodologies for accurately measuring various organic acids, which are important for assessing food quality, safety, and nutritional value. The method involved the extraction of citric acid using ultrapure water, assisted by ultrasonication, followed by quantification via High-Performance Liquid Chromatography (HPLC) with a UV-visible detector (Thermo Scientific Ultimate 3000, USA).

Approximately 1.0±0.1 g of the homogenized EBN sample was accurately weighed into a 50 mL volumetric flask. Then, 30 mL of ultrapure water were gradually added to ensure complete immersion of the sample. The flask was then subjected to ultrasonication using an ultrasonic sonicator bath (Sukinbo, China) for 10 minutes at room temperature. After ultrasonication, the volumetric flask was allowed to cool to room temperature before being brought to volume using additional ultrapure water. The solution was left undisturbed for 5 minutes to allow impurities to settle at the bottom of the volumetric flask. Then, the supernatant was syringe-filtered through a 0.45 µm nylon syringe filter membrane, discarding the initial 2 mL of filtrate to prevent contamination. The final filtrate was then collected into a 2 mL HPLC autosampler vial and stored under controlled conditions before HPLC analysis.

The chromatographic separation was performed on a Phenomenex Kinetex (United States) C18 column (150 mm × 4.6 mm, 5 µm particle size) at a column temperature of 30°C. The mobile phase consisted of 0.1% phosphoric acid:methanol (75:25, v/v), which was delivered at a flow rate of 1.0 mL/min. The detection wavelength was set at 210 nm, and the injection volume for each sample was 20 µL. A calibration curve was prepared using citric acid standard (Sigma-aldrich, Germany) solutions at concentrations of 100, 150, and 200 mg/kg. The LOD was 10 mg/kg, while the LOQ was determined at 30 mg/kg. Quality control procedures were performed as described for aluminium detection. Method validation for citric acid showed precision (RSD) value of <5% and recovery rates of 93-107%.

Statistical analysis

The data were analysed using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including mean and standard deviations at 95% confidence intervals, were calculated for both aluminium and citric acid concentrations. Normality tests were performed, and based on the results, either a t-test or Mann-Whitney U test was used to compare the results of the RUC and RC groups. In addition to significance testing, effect sizes (Cohen's *d*) were calculated, and post hoc power analysis were conducted based on observed effect size and sample sizes.

RESULTS

In this study, initial reference levels of aluminium and citric acid content in EBN were established by analysing samples from three categories: combined EBN (both RUC EBN and RC EBN), RUC EBN, and RC EBN.

Aluminium content in EBN Malaysia

Figure 2 shows the descriptive analysis of the combined EBN samples (both RUC and RC) with average aluminium content of 19.95 mg/kg, at 95% confidence interval (Cl) (Cl: 14.509 - 25.391 mg/kg). These results suggest that the overall initial reference of aluminium content for EBN should fall within this range. The median aluminium content of 19.400 mg/kg is close to the mean, indicating a relatively symmetric distribution around this central value. However, the standard deviation of 15.090 mg/kg and a wide range from non-detectable to 44.100 mg/kg indicate considerable variability in aluminium levels across the samples. The skewness value of -0.028 and kurtosis of -1.224 suggest a nearly symmetric distribution that is slightly flatter than a normal distribution.

Figure 3 shows the descriptive analysis of the RUC EBN samples and RC EBN samples separately. For RUC samples specifically, the mean aluminium content was found to be 24.176 mg/kg, with a 95% confidence interval (Cl: 15.132 - 33.219 mg/kg). The confidence interval for RUC samples suggests a slightly higher initial reference aluminium range compared to the combined data. The median aluminium content was 26.600 mg/kg, slightly higher than the mean, suggesting a slight skew

Descriptives				
			Statistic	**Std. Error**
ALUMINIUM	**Mean**		19.950	2.668
	95% Confidence Interval for Mean	**Lower Bound**	14.509	
		Upper Bound	25.391	
	5% Trimmed Mean		19.756	
	Median		19.400	
	Variance		227.717	
	Std. Deviation		15.090	
	Minimum		0.000	
	Maximum		44.100	
	Range		44.100	
	Interquartile Range		34.105	
	Skewness		-0.028	0.414
	Kurtosis		-1.224	0.809

Figure 2: Descriptive analysis of the aluminium content in EBN samples (all 32 samples)

in the distribution towards higher values. The standard deviation of 17.589 mg/kg and the range of 0 to 44.100 mg/kg indicate significant variability in aluminium content within RUC samples. Therefore, the initial reference aluminium content for RUC EBN samples can be estimated at 24.176 mg/kg, with values up to approximately 33.219 mg/kg considered within normal limits.

In contrast, the RC samples showed a lower mean aluminium content of 15.161 mg/kg, with a 95% confidence interval (Cl: 9.502 to 20.819 mg/kg). The confidence interval for RC samples defines a lower initial reference range for aluminium content compared to RUC samples. The median aluminium content was 17.700 mg/kg, slightly higher than the mean, indicating a minor left skew. With a standard deviation of 10.217 mg/kg and a range of 0.030 to 29.900 mg/kg, the RC samples displayed less variability in aluminium levels. Consequently, the initial reference aluminium content for RC EBN samples is approximately 15.161 mg/kg, with levels up to 20.819 mg/kg generally considered within normal limits.

The comparison between RUC and RC groups provided a moderate to large effect size (Cohen's d=0.62), suggesting that differences in aluminium content between these groups are not only statistically relevant but also practically meaningful. However, the achieved statistical power of this analysis was 0.39 at a significance level of α = 0.05 (two- tailed). This indicates that the present study may not have been sufficient to consistently detect differences of this magnitude, largely due to the modest sample size (N = 32). While the observed effect highlights a meaningful distinction, caution is

Descriptives				
			Statistic	Std. Error
ALUMINUM RUC	Mean		24.176	4.266
	95% Confidence Intervel for Mean	Lower Bound	15.132	
		Upper Bound	33.219	
	5% Trimmed Mean		24.412	
	Median		26.600	
	Variance		309.376	
	Std. Deviation		17.589	
	Minimum		0.000	
	Maximum		44.100	
	Range		44.100	
	Interquartile Range		39.140	
	Skewness		-0.477	0.550
	Kurtosis		-1.550	1.063
RC	Mean		15.161	2.638
	95% Confidence Intervel for Mean	Lower Bound	9.502	
		Upper Bound	20.819	
	5% Trimmed Mean		15.182	
	Median		17.700	
	Variance		104.396	
	Std. Deviation		10.217	
	Minimum		0.030	
	Maximum		29.900	
	Range		29.870	
	Interquartile Range		23.000	
	Skewness		-0.585	0.580
	Kurtosis		-0.917	1.121

Figure 3: Descriptive analysis of aluminium content of the RUC and RC samples.

warranted in interpreting these findings. Future studies with larger sample sizes are therefore recommended to strengthen the statistical reliability of aluminium comparisons between RUC and RC EBN samples.

The aluminium content in EBN samples was analysed and compared to previous studies (Table 1). For RUC house nests, this study observed aluminium levels ranging from <0.05 mg/kg (below the LOQ) to 44.10 ppm across 15 samples, which overlaps with Chen *et al*. (2014)'s range of 0.230875 to 6.437310 ppm, though our maximum concentration is notably higher. For RC house nests, our levels ranged from <0.05 mg/kg (below the LOQ) to 29.90 ppm across 14 samples, also overlapping with Chen *et al*. (2014)'s range of 0.434680 to 39.055980 ppm, but with a lower maximum value. For house nests where RUC/RC status was unspecified, Salim *et al*. (2018) reported 5.58 to 100 ppm, and Ma *et al*. (2020) found 0.002788 to 0.01344 ppm, reflecting variability possibly due to cleaning status. For RUC cave nests, this study recorded 0.49 and 38.80 ppm across 2 samples, providing new data. For RC cave nests, our value was 0.09 ppm (1 sample), compared to 3240 to 4366 ppm by Salim *et al*. (2018) and 0.01394 to 0.02330 ppm by Ma *et al*. (2020), both unspecified for RUC/RC status. The lack of cleaning status specification in some studies, along with differences in sample origins and methods, may contribute to observed variability. The results indicate variability in aluminium content across different sample types and studies, potentially due to differences in environmental exposure, processing practices, and analytical methods. Further investigation is warranted to better understand the sources and variability of aluminium in EBN.

Table 1. Comparison of aluminium content in EBN samples (ppm) with other researches

	Chen *et al.,* 2014	Salim *et al.,* 2018	Ma *et al.,* 2020	This study
RUC house nest	0.230875-6.437310	N/A	N/A	< 0.05–44.10 (n = 15)
RC house nest	0.434680-39.055980	N/A	N/A	< 0.05-29.90 (n = 14)
House nest	N/A	5.58 – 100*	0.002788- 0.013440*	N/A
RUC cave nest	N/A	N/A	N/A	0.49 & 38.80 (n =2)
RC cave nest	N/A	N/A	N/A	0.09 (n = 1)
Cave nest	N/A	3240.00 - 4366.00*	0.013940- 0.023300*	N/A

Notes: *Not specified whether RUC or RC samples; N/A – not applicable.

Table 2. Percentage (number) of samples detected to contain aluminium according to concentration in comparison with data from Explanation of the Standard Compilation for Edible Bird's Nest (Dried Products) (Draft for Public Comment) (China National Food Industry Association, 2024)

Concentration in mg/kg	Data from the Explanation of the Standard Compilation for Edible-Birdnest (Dried Products) (Draft for Public Comment)	This study (RUC)	This study (RC)
>100	12.70% (n=20)	0.00% (n=0)	0.00% (n=0)
25-100	25.30% (n=40)	58.82% (n=10)	13.33% (n=2)
10-25	25.30% (n=40)	11.76% (n=2)	60.00% (n=9)
1.0-10	31.60% (n=50)	0.00% (n=0)	0.00% (n=0)
<0.05-1.0	5.10% (n=8)	23.53% (n=4)	26.67% (n=4)
Detected	79.00% (158/200)	94.12% (16/17)	100% (15/15)

Citric acid content

The descriptive statistical analysis of combined citric acid concentrations (Figure 4) in EBN samples (N=32) revealed a mean value of 4079.463 mg/kg, at 95% confidence interval (Cl: 3179.697 - 4979.228 mg/kg). The median concentration was recorded as 4007.500 mg/kg, which closely aligns with the 5% trimmed mean of 4006.747 mg/kg, indicating minimal influence from outliers on the central tendency. The citric acid concentrations exhibited a wide range, from a minimum of 328.100 mg/kg to a maximum of 9719.600 mg/kg, resulting in a total range of 9391.500 mg/kg. This variability highlights the substantial differences in citric acid levels across the samples, potentially influenced by factors such as geographical origin, swiftlet house condition, processing methods, or environmental conditions. The interquartile range (IQR) of 3726.100 mg/kg further reflects the dispersion of data around the central values. The standard deviation of 2495.616 mg/kg and variance of 6,228,098.890 indicate a high degree of variability in the citric acid concentrations among the samples. The skewness value of 0.161 suggests that the data distribution is slightly positively skewed, though nearly symmetric, while the kurtosis value of -0.567 indicates a flatter distribution compared to a normal curve.

Figure 5 shows the descriptive statistical analysis of citric acid concentrations in RUC and RC EBN samples revealed significant differences between the two groups. For RUC samples (N=17), the mean citric acid concentration was 5492.282 mg/kg, at 95% confidence interval (Cl: 4295.919 - 6688.646 mg/kg). The median concentration was higher at 5786.300 mg/kg, reflecting slightly higher values in the dataset. The minimum concentration recorded was 328.100 mg/kg, while the maximum reached 9719.600 mg/kg, resulting in a total range of 9391.500 mg/kg. The variability in RUC samples was evident from the standard deviation of 2326.865 mg/kg and an interquartile range (IQR) of 2825.750 mg/kg, highlighting substantial dispersion around the mean. The skewness value of -0.631 and kurtosis of 0.881 indicate a slightly left-skewed and flatter distribution compared to normal distribution.

			Statistic	Std. Error
CITRIC ACID	**Mean**		4079.463	441.167
	95% Confidence Intervel for Mean	**Lower Bound**	3179.697	
		Upper Bound	4979.228	
	5% Trimmed Mean		4006.747	
	Median		4007.500	
	Variance		6228098.890	
	Std. Deviation		2495.616	
	Minimum		328.100	
	Maximum		9719.600	
	Range		9391.500	
	Interquartile Range		3726.100	
	Skewness		0.161	0.414
	Kurtosis		-0.567	0.809

Figure 4: Descriptive statistical analysis of combined citric acid concentrations in EBN samples

In contrast, for RC samples (N =15), the mean citric acid concentration was markedly lower at 2478.267 mg/kg, at 95% confidence interval (Cl: 1614.603 - 3341.930 mg/kg). The median concentration was 3175.700 mg/kg, indicating a less dispersed dataset compared to RUC samples. The citric acid concentrations in RC samples ranged from a minimum of 408.500 mg/kg to a maximum of 5188.000 mg/kg, with a total range of 4779.500 mg/kg. The standard deviation of 1559.574 mg/kg and an interquartile range (IQR) of 2995.200 mg/kg suggest lower variability compared to RUC samples. The skewness value of -0.631 and kurtosis of 0.881 for RC samples suggest a distribution that is nearly symmetrical and flatter than normal.

	Descriptives				
GROUP		**Measure**		**Statistic**	**Std. Error**
CITRICACID	**RUC**	**Mean**		5492.282	564.348
		95% Confidence Intervel for Mean	**Lower Bound**	4295.919	
			Upper Bound	6688.646	
		5% Trimmed Mean		5544.330	
		Median		5786.300	
		Variance		5414300.657	
		Std. Deviation		2326.865	
		Minimum		328.100	
		Maximum		9719.600	
		Range		9391.500	
		Interquartile Range		2825.750	
		Skewness		-0.631	0.550
		Kurtosis		0.881	1.063
	RC	**Mean**		2478.267	402.680
		95% Confidence Intervel for Mean	**Lower Bound**	1614.603	
			Upper Bound	3341.930	
		5% Trimmed Mean		2442.713	
		Median		3175.700	
		Variance		2432272.480	
		Std. Deviation		1559.574	
		Minimum		408.500	
		Maximum		5188.000	
		Range		4779.500	
		Interquartile Range		2995.200	
		Skewness		-0.105	0.580
		Kurtosis		-1.239	1.121

Figure 5: Descriptive analysis of citric acid content of the RUC and RC samples.

The comparison between RUC and RC groups revealed a very large effect size of citric acid (Cohen's *d* = 1.42), indicating a strong and practically meaningful difference between the two groups. The achieved statistical power for this analysis was 0.98 at a significance level of α=0.05 (two-tailed), demonstrating that the sample size (N=32) was more than adequate to detect the observed effect. These findings strengthen the evidence that citric acid levels differ substantially between RUC and RC EBN samples, with the higher values in RUC samples reflecting potential impacts of raw material condition and the influence of cleaning and processing procedures, which could reduce the citric acid content in EBN. The high power of the analysis provides confidence in the robustness of this result. The data provides a comprehensive initial reference for citric acid levels in EBN, contributing to the understanding of how processing and sample types affect the natural composition of this compound.

Comparison of RUC and RC

The statistical analysis was conducted on 15 paired samples, representing EBN samples before (RUC) and after (RC) undergoing the cleaning process. The paired-sample approach allows for a direct comparison of the changes in aluminium and citric acid content, within the same batch of samples. This methodology provides a robust framework for evaluating the effectiveness of the cleaning process in reducing potentially harmful substances. By analysing paired samples, external variability is minimized, ensuring that observed differences are attributed solely to the cleaning intervention.

To confirm the distribution pattern of aluminium content in each group, the Shapiro-Wilk test for normality was performed. The results showed a Shapiro-Wilk statistic of 0.817 for RUC samples with a p-value of 0.003, indicating that aluminium content in RUC samples does not follow a normal distribution. Similarly, the RC samples had a Shapiro-Wilk statistic of 0.858 with a p-value of 0.022, also suggesting non-normal distribution for aluminium content in RC samples. Given these results, both RUC and RC aluminium content data deviate significantly from a normal distribution. The Wilcoxon Signed-Rank Test was selected to evaluate the differences in aluminium content between RUC and RC EBN samples due to the non-normal distribution of the data (Figure 6), and analysing paired data to determine the effect of the cleaning process on aluminium levels in the samples. The results suggest that the cleaning process significantly reduces aluminium content in EBN samples. The aluminium content in RC samples is significantly lower compared to RUC samples, as indicated by the p-value of 0.012.

Related-Sample Wilcoxon Signed Rank Test Summary	
Total N	15
Test Statistic	16.000
Standard Error	17.607
Standardized Test Statistic	-2.499
Asymptotic Sig.(2-sided test)	0.012

Figure 6: Results of the Wilcoxon Signed-Rank Test for aluminium content in RUC and RC EBN samples.

The tests of normality for the citric acid content of the two groups: RUC and RC EBN samples were performed. For the RUC group, the p-values for both the Kolmogorov-Smirnov (0.200) and Shapiro-Wilk (0.523) tests are greater than 0.05. This indicates that the citric acid content for the RUC samples does not significantly deviate from a normal distribution. Similarly, for the RC group, the p-values for both the Kolmogorov-Smirnov (0.087) and Shapiro-

Wilk (0.102) tests are also greater than 0.05. This suggests that the citric acid content for the RC samples is normally distributed. Based on the results of both normality tests, the citric acid content for both the RUC and RC groups follows a normal distribution. Therefore, parametric statistical tests (e.g., paired t-test or independent samples t-test) may be appropriate for comparing citric acid levels between the two groups. The results (Figure 7) showed a statistically significant difference (<0.01) between the citric acid content of RUC and RC samples. These findings confirm that the cleaning process effectively reduces citric acid levels in EBN. The results highlight the importance of cleaning as a critical step in processing EBN, particularly for ensuring compliance with food safety standards and enhancing the quality of the final product.

The comparative analysis between RUC and RC EBN samples highlights the significant impact of the cleaning process on reducing aluminium and citric acid content. Statistical evaluations revealed a substantial reduction in aluminium levels post-cleaning, as evidenced by the Wilcoxon signed-rank test, confirming the cleaning process's efficacy in mitigating potential aluminium contamination. Similarly, the paired-samples t-test demonstrated a marked decrease in citric acid content, further indicating the effectiveness of cleaning in altering the chemical composition of EBN. These findings underscore the critical role of the cleaning process in improving the safety and quality of EBN by reducing the concentrations of potentially harmful substances.

Initial Reference Values for Aluminium and Citric Acid Content

This study establishes initial reference values for aluminium and citric acid content in EBN samples, providing a critical starting point for evaluating the natural composition of EBN and the effects of processing. For combined EBN samples (N=32), the mean aluminium content was 19.950 mg/kg, with an upper reference range extending to 25.391 mg/kg. The aluminium content in RUC EBN samples was higher, with a mean value of 24.176 mg/kg and a reference range up to 33.219 mg/kg, while RC EBN samples exhibited a lower mean aluminium content of 15.161 mg/kg, with a reference range up to 20.819 mg/kg. Similarly, for citric acid content, the mean concentration for combined EBN samples was 4079.463 mg/kg, with an upper reference range extending to 4979.228 mg/kg. In comparison, the mean citric acid content for RUC EBN samples (N=17) was 5492.282 mg/ kg, with a reference range up to 6688.646 mg/ kg, whereas RC EBN samples (N=15) exhibited a significantly lower mean citric acid content of 2478.267 mg/kg, with a

Paired Simple Test								
Paired Differences								
				95% Confidence Interval of the Different				
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1 CITRICACIDRUC - CITRICACIDRC	3365.433	1914.885	494.421	2305.006	4425.861	6.807	14	0.000

Figure 7: Paired-Samples T-Test results for citric acid content in RUC and RC EBN samples.

reference range up to 3341.930 mg/kg. The results indicate that both aluminium and citric acid concentrations are higher in RUC samples compared to RC samples, highlighting the impact of cleaning and processing in reducing these components. These initial reference values are vital for understanding the compositional variability of EBN, supporting quality control, regulatory compliance, and guiding future research on EBN safety and processing.

DISCUSSION

Environmental contamination during nest formation in birdhouses or caves largely explains the presence of aluminium in RUC EBN samples. Several potential sources of aluminium contamination include airborne particles, water contamination, and construction materials used in birdhouses. Airborne aluminium particles from surrounding industrial activities, soil, or natural dust can settle on the nests, leading to external contamination (Agency for Toxic Substances and Disease Registry (ATSDR), 2008; Wang *et al*., 2007). Similarly, water (unfiltered) used for cleaning or to maintain humidity in birdhouses may contain aluminium (Mng'ong'o & Matimbwa, 2025; Nur Fahirah *et al*., 2019; Qaiyum *et al*., 2011), particularly if treated with aluminium sulfate (alum) for clarification (Tahraoui *et al*., 2024). Residual aluminium in such water may transfer to EBN during cleaning or maintenance.

Dietary pathways may also contribute. Insects, the primary food source for swiftles, bioaccumulate heavy metals including aluminium with concentrations reported up to 5000 mg/kg (Sparling & Lowe, 1996), highlighting their capacity to bioaccumulate metals. Metal accumulation insects vary with species, developmental stage and environment (Malematja *et al*., 2023). While direct studies on aluminium transfer from insects to swiftles EBN are lacking, similar pathways have been documented for other trace elements in avian species (Wahyuni *et al*., 2022), suggesting this is a plausible mechanism that warrants further investigation. These combined pathways likely explain the variability in aluminium levels observed across RUC samples.

Table 2 shows the aluminium content in EBN samples from this study was categorized into different concentration ranges and compared to the values presented in the "Explanation of the Standard Compilation for Edible Bird's Nest (Dried Products) (Draft for Public Comment)" (China National Food Industry Association, 2024). The draft standard was initiated in September 2023 by the China National Food Industry Association as part of its effort to standardize the safety and quality of EBN products. This initiative aimed to address increasing concerns about food safety, particularly with respect to contaminants such as aluminium, in EBN imported into China from various countries, including Malaysia and Indonesia. The draft standard was developed with significant contributions from several organizations and companies. The primary drafting entities included Xiamen Yan Zhi Wu Silk Biotech Co., Ltd. and Yan Zhi Wu Healthy Beauty (Xiamen) Food Co., Ltd. These companies led the technical drafting process, working closely with the China National Food Industry Association to compile initial reference data on EBN quality and contamination levels. The draft evaluated 200 EBN samples from multiple exporting countries and identified aluminium as a key safety parameter. Results showed that 79% of the samples contained detectable aluminium, with concentrations ranging from below 0.05 mg/kg to 1500 mg/kg. Based on these findings, the maximum allowable aluminium concentration for dried EBN products was set at 100 mg/kg (on a dry weight basis), referencing the existing Chinese National Food

Safety Standard: Standards for the Use of Food Additives (2014), GB 2760-2014. Although GB 2760-2024 has long been applied to various foods, its enforcement was extended to cover EBN products on 8 February 2025, marking the first time this regulation has been implemented for the bird's nest export sector.

In contrast, this study of 32 Malaysian samples found maximum concentrations of 44.1 mg/kg (RUC) and 29.9 mg/kg (RC), with none exceeding the 100 mg/kg threshold. These results suggest that Malaysian production practices may limit contamination more effectively than in other regions. Importantly, the new requirement under China's GB 2760-2024 standard—implemented for EBN products on 8 February 2025—also sets a maximum allowable aluminium concentration of 100 mg/kg (dry weight basis), and all Malaysian samples in this study complied with this limit. Furthermore, comparison with EFSA's tolerable weekly intake (TWI) of 1 mg/kg body weight and JECFA's provisional tolerable weekly intake (PTWI) of 2 mg/kg body weight indicates that typical EBN consumption remains within international safety limits, although excessive intake could pose risks for vulnerable populations. This study suggests that modern processing practices in Malaysia may have effectively reduced contamination risks. The distribution of aluminium concentrations in both datasets emphasizes the variability in contamination levels across EBN types and sources. Higher aluminium levels in the draft standard samples may be attributed to external contamination sources, regional differences in environmental exposure, or differences in regulatory compliance during processing.

Based on the aluminium content measured in combined EBN samples (mean: 19.95 mg/kg, 95% CI: 14.51–25.39 mg/kg), the estimated aluminium intake from consuming EBN can be assessed. Assuming a consumer weighs 40 kg and consumes EBN weekly, the PTWI established by JECFA would allow an intake of up to 80 mg of aluminium per week (2 mg/kg × 40 kg), while EFSA's stricter TWI would permit a maximum of 40 mg per week (1 mg/kg × 40 kg). For RUC EBN samples, with an average aluminium content of 24.18 mg/kg (95% CI: 15.13–33.22 mg/kg), a weekly consumption of approximately 3.3 kg would approach JECFA's PTWI, while only 1.7 kg would exceed EFSA's TWI. In contrast, for RC EBN samples, with an average aluminium content of 15.16 mg/kg (95% CI: 9.50–20.82 mg/kg), the weekly consumption thresholds increase to 5.3 kg for JECFA's PTWI and 2.6 kg for EFSA's TWI. These calculations demonstrate that while the aluminium levels in EBN are generally within safe limits for typical consumption quantities, they may raise concerns for individuals consuming unusually large amounts or for vulnerable populations, such as children or individuals with impaired renal function. Establishing initial reference values such as those provided in this study can help EBN producers in Malaysia monitor and regulate aluminium levels in their products.

The potential reduction of aluminium content in EBN through cleaning processes can be attributed to several mechanisms, although specific data on aluminium reduction is limited. The reduction of heavy metal concentrations in EBN during cleaning has been reported in previous studies (Wahyuni *et al*., 2022), suggesting that at least some of the aluminium present is in a water-soluble form that can be effectively removed during washing. The dissolution and removal of these contaminants in wash water are believed to significantly contribute to the overall reduction of aluminium content in cleaned EBN. The primary cleaning process, which involves sorting, surface cleaning, and impurity removal (Yeo *et al*., 2021), likely reduces surface-bound aluminium originating from environmental sources such as dust or particulates. Furthermore, using well-filtered potable water for aluminium removal in the cleaning process may facilitate the removal of water-soluble aluminium compounds. The elimination of impurities such as feathers, eggshells, dirt, paint, wood, twigs, cement, sand, and soil during cleaning (Xuan *et al*., 2023) likely contributes to overall aluminium reduction, as these materials may contain aluminium. Extended water exposure during cleaning, sometimes lasting 2-4 hours (Azmi *et al*., 2021), may promote the leaching of soluble aluminium salts from the EBN structure, potentially reducing the overall aluminium content. However, it is important to note that while these cleaning processes likely reduce various contaminants, including potentially aluminium, the specific impact on aluminium content is not directly addressed in current literature. This study represents the first comprehensive investigation targeting aluminium reduction during the EBN cleaning process by analysing pre and post cleaning aluminium content. While these findings highlight the effectiveness of the cleaning process in reducing aluminium, further research is necessary to explore the specific aluminium species present in EBN and their solubility characteristics. Such studies would help to better understand the role of different cleaning methods and quantify their efficacy in aluminium removal.

Citric acid levels in EBN are influenced by biological, microbial, and environmental factors. Insects consumed by swiftlets may ingest plant material rich in citric acid, leading to indirect incorporation into EBN via saliva. Microbial activity may also contribute: fungi such as *Aspergillus niger* (commonly used for industrial production) and the bacterial such as *Bacillus licheniformis*, both detected in EBN samples (Chen *et al*., 2015; Wong *et al*., 2018), are known to produce citric acid as a metabolic by-product. Environmental pollution, particularly in regions with industrial activities or agricultural practices that utilize citric acid, may lead to its deposition on the nests. Understanding the sources and mechanisms underlying citric acid presence in RUC EBN is essential for interpreting its variability and implications. Future studies should focus on quantifying contributions from these potential sources and their impacts on EBN composition to ensure better control and standardization during processing.

This study shows the significant reduction of citric acid content in EBN following primary processing or cleaning can be attributed primarily to two mechanisms: the high-water solubility of citric acid, which facilitates its dissolution and removal during washing and soaking steps, and the physical removal of citric acid-containing contaminants through mechanical cleaning processes such as scrubbing or brushing. The extent of this reduction underscores the need for further research, for example developing standardized processing protocols that strike a balance between citric acid reduction and the preservation of other beneficial components is essential for ensuring consistent product quality across the industry.

The results of this study also revealed mean citric acid concentrations of 4079.46 mg/kg for combined EBN samples, with higher levels observed in RUC samples (5492.28 mg/kg) compared to RC samples (2478.27 mg/kg). While these values exceed the limits set for certain ready-to-eat food categories, it is critical to consider the impact of cooking processes on citric acid levels. According to Chan *et al*. (2015), citric acid content in EBN decreases by approximately 90% following standard thermal processing.

This significant reduction is attributed to citric acid primarily existing in a free salt form, which is highly susceptible to degradation during cooking. Consequently, the high citric acid levels detected in RUC and RC EBN samples are likely not reflective of the final concentrations in prepared EBN products consumed by end-users. These findings emphasize the importance of considering EBN's unique preparation requirements when evaluating compliance with regulatory standards. While primary cleaning processes effectively reduce citric acid content, additional reductions during cooking further mitigate concerns about excessive citric acid levels. Establishing EBN-specific food safety guidelines that account for its preparation and cooking processes, alongside regular monitoring of citric acid content in both raw and prepared products, will ensure compliance with international standards and maintain consumer safety. Such tailored guidelines will support Malaysian EBN producers in meeting food safety expectations and expanding their presence in global markets.

This comprehensive evaluation of aluminium and citric acid levels in EBN highlights the critical role of cleaning and processing in ensuring product safety, reinforcing the need for stringent quality control and regulatory alignment. This study has some limitations. First, the sample size, while representative of multiple regions in Malaysia, remains modest and may not capture all sources of variability, particularly for cave nests. Second, the study did not account for regional and seasonal differences, which may influence aluminium and citric acid levels through variations in environmental exposure, water quality, or swiftlet diet. Third, the analysis focused only on aluminium and citric acid, while other potential contaminants such as nitrites or microbial hazards were not assessed. Finally, cooking simulations were not performed, so post-processing reductions of citric acid were inferred from published literature rather than directly measured. Future research should expand further on these limitations to ensure a more comprehensive report on the safety of EBN consumption as healthy food.

CONCLUSION

This study provides the first comprehensive analysis of aluminium and citric acid content in Malaysia EBN, directly addressing concerns raised by China Customs. Initial reference values were established for both RUC and RC samples, with RUC showing higher aluminium (24.18 mg/kg) and citric acid (5492.28 mg/kg) concentrations compared to RC (15.16 mg/kg and 2478.27 mg/kg, respectively). The results confirm that cleaning significantly reduces both components, underscoring its importance in enhancing product safety and quality.

Most importantly, all Malaysian samples complied with the newly implemented China GB 2760 standard for EBN (100 mg/kg aluminium limit, effective 8 February 2025), as well as international benchmarks set by EFSA and JECFA, highlighting Malaysia's regulatory readiness. While aluminium generally falls within safe limits for typical consumption, risks may remain for excessive intake or vulnerable populations. Similarly, high citric acid levels observed in raw samples are likely to be further reduced by cooking, consistent with published evidence.

In conclusion, this research provides valuable preliminary data for aluminium and citric acid content in Malaysian EBN, contributing to a better understanding of EBN composition and the effects of processing. The findings underscore the importance of proper cleaning and processing techniques in ensuring EBN safety and quality. To complement this study limitations, future research should expand sampling across regions and seasons, examine additional contaminants, and experimentally evaluate the effects of cooking on aluminium and citric acid reduction. Developing standardized cleaning and processing protocols, tailored food safety guidelines for EBN, and effective monitoring systems will be critical to ensure product safety and strengthen Malaysia's position in the global EBN market.

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