# Structural analysis of raw and commercial farm edible bird nests

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Abstract. Edible bird nests (EBNs) are consumed worldwide for various health benefits. EBNs are nests built from the saliva of swiftlets of Aerodramus species. The global market for EBNs is on the rise, especially from Hong Kong and mainland China. In the past, EBNs were harvested mainly from natural caves; however in the recent years, there has been a rapid growth of swiftlet farming. Little is known about the actual composition of EBNs except for protein, carbohydrate, ash and lipid contents, amino acids, vitamins and macro/ micronutrients. Besides the biochemical components of EBNs, are there any other structures that are associated with EBNs? This paper reports on the structural analysis of raw unprocessed farm and processed commercial EBNs. The raw EBNs were purchased from swiftlet farms in five locations in Peninsula Malaysia: Kuala Sanglang (Perlis; 6° 16' 0"N, 100° 12' 0"E), Pantai Remis (Perak; 4º 27' 0" N, 100° 38' 0" E), Kluang (Johor; 02° 012 303N 103° 192 583E), Kajang (Selangor; 2° 59' 0"N, 101° 47' 0"E) and Kota Bharu (Kelantan; 6° 8' 0"N, 102° 15' 0"E). The commercial nests were purchased from five different Chinese traditional medicinal shops (Companies A-E). A portion of each EBN was randomly broken into small fragments, attached to carbon tape and coated with gold and palladium particles for examination and photography under a scanning electron microscope. Structural analysis revealed the presence of mites, fungi, bacteria and feather strands on both the raw and commercial nests. Mite eggshells and faecal pellets, and body parts of other arthropods were seen only in the raw nests. The commercial nests had a variety of unidentified structures and substances coated on the nests' surfaces that were not found on the raw nests. The presence of these contaminants may jeopardise the quality of EBNs and pose health risks to consumers. Further identification of the mites and their allergens, fungi and bacteria are on-going and will be reported separately.

### INTRODUCTION

For centuries, edible bird nests (EBNs) have been consumed especially by the Chinese in the form of double-boiled soup with rock sugar. Today, EBN is still consumed as a delicacy for its perceived medicinal properties and health benefits. EBN is the dried form of the nests made from the regurgitated saliva of four different species of swiftlets of the genus *Aerodramus* (formerly called *Collocalia*). Raw or unprocessed EBNs are harvested from natural caves (cave nests) and swiftlet farms or abandoned shops in town (house/farm nests). The raw nests go through the process of soaking, cleansing, bleaching, moulding and packaging before they are sold (Ma & Liu, 2012).

The global demand for EBNs especially from Hong Kong and mainland China is increasing. The swiftlet farming industry has been expanding over the past decades, providing ample economic opportunities for the producer countries in Southeast Asia (Lim, 2011), including Malaysia. Malaysia is the second largest exporter of EBNs where eighty percent of the EBNs are exported to China. In 2011, there was a scare over high levels of nitrites in the EBNs exported by Malaysia, causing China to ban the import of EBNs from Malaysia. The ban was lifted in November 2011. In February 2012, EBNs (cubilose; except for canned version) were again listed as banned products by the Ministry of Agriculture and the General Administation of Quality Supervision, Inspection and Quarantine, China (Zhang, 2012). Thus the food safety aspects of EBNs are crucial to the viability of the Malaysian EBN industry.

So far, the benefits, nutritional and non-nutritional contents of EBNs remain undetermined. EBN is composed of protein (62–63%), carbohydrate (25.62–27.26%), ash (2.1%) and lipid (0.14–1.28%) (Marcone, 2005). EBN also contains minerals (the top four are calcium, sodium, magnesium and potassium). Sometimes, in order to increase the weight of the commercial EBNs, adulterants (karaya gum, red seaweed and tremella fungus) are added. They typically account for 2–10% of the nests (Marcone, 2005).

There have been reports of allergic symptoms and food-induced anaphylaxis following the ingestion of EBNs. EBN was the common cause of food-related anaphylaxis among the children (Goh et al., 1999; Kemp et al., 2010) and adults (Thong et al., 2005; 2007) in Singapore. In Hong Kong, children developed moderate-to-severe atopic eczema after consuming EBN soup and traditional Chinese medicine (Hon et al., 2006). Generalised urticaria and anaphylactic shock were reported in paediatric cases following the consumption of EBN drinks (Hon et al., 2009). In a retrospective study, 23 (92%) of 25 patients who were previously identified as having probable EBN-induced anaphylaxis, had positive Food Allergy Specific Immunotherapy (FAST), skin prick test (SPT), or both when tested with crude extracts of unprocessed EBN from Sarawak (Goh et al., 2000). IgE binding to extracts of commercial EBNs was reported in those subjects who were allergic to EBNs (Goh et al., 2001). Obviously, there may be many more unreported EBN-related food allergies in other countries. Immuno-characterisation of sera from patients with allergic and anaphylactic reaction to EBNs revealed the potential allergens of 66 kDa, 77 kDa, and 100 kDa (Goh *et al.*, 2001; Marcone, 2005). The 66 kDa and 77 kDa allergens share sequence homology with the egg white allergens ovoinhibitor and ovotransferrin respectively.

However, the source(s) of these allergens remains unknown. The possible source(s)may originate from the saliva or feathers of the swiftlets, the insects ingested by the swiftlets, the environment, the microorganisms associated with the nests (such as fungi, bacteria and protozoa), arthropods (such as mites) which inhabit the swiftlets or their nests, the cleaning processes of the raw nests, the adulterants added to the commercial nests and/or the contaminants introduced, and the infestation of arthropods or other organisms during the storage of the nests. Hence, this study was designed to examine the raw unprocessed farm and processed commercial EBNs and any other associated structures on the EBNs.

### MATERIALS AND METHODS

The unprocessed (raw and un-cleaned) EBNs were purchased from house farms in five different localities in Malaysia: Kuala Sanglang (Perlis; 6° 16' 0" North, 100° 12' 0" East), Pantai Remis (Perak; 4° 27' 0" North, 100° 38' 0" East), Kluang (Johor; 02° 012 303 North 103° 192 583 East), Kajang (Selangor; 2° 59' 0" North, 101° 47' 0" East) and Kota Bharu (Kelantan; 6° 8' 0" North, 102° 15' 0" East). The commercial EBNs were purchased from five different Chinese traditional medicine shops (Companies A-E). Three to six nests were purchased from each locality/ shop. The nests were sealed in a plastic bag and transported to the laboratory.

Upon arrival at the laboratory, a portion of the nest was randomly broken off, pressed or gently crushed into tiny fragments, placed on the stage with carbon tape, coated with gold and palladium particles (1:3) and was examined carefully under a scanning electron microscope (TM3000, Hitachi, Japan). The structures of the EBNs, the mites, faecal pellets and other contaminants were then examined and photographed.

## RESULTS

Under the SEM, a number of structures were seen on the surface of the raw nests including mites (Figs. 1a-c), mite eggshells (Figs. 1d & e), faecal pellets (Fig. 1f), other arthropods (Fig. 2a), bacteria (Fig. 2b), yeast or fungal spores (Figs. 2d-f), feather strands (Fig. 3a), and few unidentified structures (Figs. 3b-f). The mites were caught among the feather strands (Fig. 1a), partly embedded within the nest (Fig. 1b), or on the surface of the nest (Fig. 1c). The mite eggshells found had microsculpture protruding out of their surfaces (Figs. 1d & e).

Certain broken parts like leg, abdomen and mouthpart of other arthropods were seen (Fig. 2a). The fungal structures found on the surface of the raw EBNs included yeast and hyphae (Fig. 2c) and various kinds of fungal spores (Figs. 2d-f).

Similarly, mites (Figs. 4a & b), bacteria (Fig. 4c), fungal structures (Figs. 4d-f) and feather strands (Fig. 5a) were also found on the surface of the commercial EBNs (Table 1). Most of the mites found (Figs. 4a & b) were partially embedded within the nest. Sometimes, a few mites were clumped together in the EBN crevices (figure not shown). Fungal structures observed included hyphae (Figs. 4e & f), yeasts (figure not shown) and fungal spores (Fig. 4d). Surprisingly, even after commercial processing, feather strands were still found in the commercial EBNs (Fig. 5a); with some of them partially embedded in the EBNs. Some feathers still maintained their structures whereas others seemed to have lost their natural structures.

There was a very wide variety of unidentified defined structures found in the commercial nests (Figs. 5b-i). These structures seemed to be coated with a layer of partially clear/transparent substance. Substances which appeared rough and hard covered some of the surfaces on the commercial EBNs (Fig. 5g) and were similar to those observed on the surface of the raw EBNs (Fig. 3f). However, other than these, all the other unidentified structures found in the commercial nests were not seen in the raw nests.

## DISCUSSION

EBNs are widely consumed in various parts of the world. These nests are built from the saliva of the swiftlets. However, the nutritional contents or active ingredients and allergenic components of this delicacy still remain undetermined. Structural analysis of both raw and commercial EBNs revealed the presence of mites and their faecal pellets. Mites have been associated with birds and their nests; in fact, every bird has its own mite fauna except for the penguin (Proctor & Owens, 2000). A high number of mites were isolated from the nests of birds, e.g. those found in the nests of house finches (Carpodacus mexicanus) (Stoehr et al., 2000). This is probably due to Malaysia's tropical climate which is conducive for mite propagation. Higher mite density was reported in tropical countries compared with temperate countries due to favourable conditions that enable the mites to complete at least 12 cycles of development a year, building up huge populations (Nadchatram, 2005).

More contaminants were found on the raw EBNs compared with processed commercial EBNs especially for the mite faecal pellets and mite eggshell. This could be due to their removal during the cleaning process of the commercial nests. During the process, the nests are soaked in a large amount of water overnight in a container. The large feathers are removed with tweezers. After squeezing out excess water from the nests, a small amount of oil is added. The nests are then mechanically stirred and rotated for about 15 minutes in order to separate the strands of the nest from fine feathers and other debris. Then, the nests are poured back into the large container filled with large amount of water. The feathers, mite faecal pellets, eggshells, mites and other lighter structures may float and be coated with oil and be removed when the water is being poured away. The long strands of nests are then collected, shaped into shape and dried in an oven as premium grade nests while the broken strands are meshed to form nest ball or disc (Ma & Liu, 2012).



Figure 1. Scanning electron micrographs of mite structures found on the raw EBNs. (a) Mite trapped among the feather strands and debris; (b & c) Mite(s) embedded in or on the surface of the EBN; (d & e) Hatched mite eggshells with defined microsculptures; (f) mite faecal pellets



Figure 2. Scanning electron micrographs of (a) abdominal cuticle of other arthropods; (b) Streptococci bacteria; (c) budding yeasts and hyphae; (d-f) different types of fungal spores found on the raw EBNs. Circles indicate the fungal spores. Arrow indicates the location of the streptococcal bacteria



Figure 3. Scanning electron micrographs of (a) feather strands; and (b-f) other unidentified structures found on the raw EBNs



Figure 4. Scanning electron micrographs of (a & b) mites; (c) rod shaped bacteria; and (d-f) fungal elements or spores found on the commercial EBNs. Arrows indicate the locations of the fungal elements or spores



Figure 5. Scanning electron micrographs of (a) feather strands; (b-i) unidentified structures found on the commercial EBNs. Note structure (h) resembled karaya gum

Structures found	Raw EBNs	Commercial EBNs
Mites	$\checkmark$	$\checkmark$
Mite eggshells	$\checkmark$	
Faecal pellets	$\checkmark$	
Other arthropods	$\checkmark$	
Fungal structures	$\checkmark$	$\checkmark$
Bacteria	$\checkmark$	$\checkmark$
Feather strands	$\checkmark$	$\checkmark$
Others	$\checkmark$	$\checkmark$

Table 1. Summary of structures seen in the raw and commercial EBNs under SEM

Despite the washing and processing of the raw EBNs, mites and strands of feathers were still found on the surface of the commercial nests and also embedded within the strands of EBNs. Those embedded within the strands of the nests were only visible after breaking the nest into small pieces. The amount of mites seen in the nests differs among companies and this may reflect the stringency and thoroughness of the cleaning processes applied to the raw nests. Hence, standardisation of cleaning method and quality checks should be put in place in order to eliminate public concerns on food-induced anaphylaxis subsequent to ingestion of mite contaminated EBNs.

In recent years, there have been more reports relating the consumption of EBNs with the development of food-induced allergy and anaphylaxis. These cases may be attritubed to many mite and microbial components observed in the present study. Many anaphylaxis cases following the ingestion of mite-contaminated food have been reported over the years. Oral mite anaphylaxis is a life-threatening condition as when the patients experienced acute respiratory failure due to laryngeal oedema following the consumption of mite-infested food (Sanchez-Borges et al., 2005). Case reports implicated house dust and storage mites as the aetiology of oral mite anaphylaxis, e.g. pancakes made from flour contaminated with Dermatophagoides farinae and Blomia freemani (Erben et al., 1993; Wen et al., 2005), grated bread containing Suidasia sp. (Sanchez-Borges et al., 1997), fried fish coated with Japanese flour contaminated with D. farinae and scones baked from flour contaminated with D. farinae (Tay et al., 2008). The other mites that have been associated with oral mite anaphylaxis include Dermatophagoides pteronyssinus, Tyrophagus sp. and Aleuroglyphus ovatus, which are also house dust and storage mites (Sanchez-Borges et al., 2012). Mites have been reported to be present in the stool and urine samples of the sensitised subjects. These include Acarus siro, Tyrophagus putrescentiae, Tyrophagus longior, D. farinae, D. pteronyssinus, Glycyphagus domesticus, G. ornatus, G. domesticus, Carpoglyphus lactis, Tarsonemus granaries, Tyrophagus granarus, Tyrophagus hominis, A. ovatus, Caloglyphus berlesei, Caloglyphus mycophagus, Caloglyphus hughesi, Suidasia nesbitti, Lardoglyphus konoi, Lepidoglyphus destructor and Euroglyphus *magnei*. These mites caused intestinal acariasis (Chao et al., 2003). Approximately 198 to 320 mites in 100 mg of flour were reported to cause life threatening anaphylaxis (Matsumoto, et al., 2001; Matsumoto & Satoh, 2004). Hence, the density of mites associated

with these EBNs needs to be determined and will be reported separately.

Mites have been known to be a rich source of allergens; nearly 130 mite allergens have been identified (Colloff, 2009a). It has been suggested that mite specific IgE is responsible for mediating oral mite anaphylactic reactions. Some of the mite allergens are enzymes (e.g. serine proteases and cysteine proteases) that cause direct damage to the airway and gastrointestinal mucosa. The introduction of mite allergens through the gastrointestinal mucosa following ingestion may either induce immunological tolerance, or result in enhanced sensitisation due to long-term exposure to low amounts of the same allergen. Such sensitisation is liable to cause a systemic allergic reaction, as seen in oral mite anaphylaxis, following the consumption of high quantities of allergen (Sanchez-Borges et al., 2005). Exposure to a level above the individual tolerable or threshold level of allergen concentration will lead to the development of allergy. However, studies have also suggested that the development of sensitisation and disease is much more complex and multifactorial than a simple linear dose-response relationship between allergen concentration and development of allergy (Colloff, 2009b).

Consumers purchase not only the commercial nests, but also the raw nests as they are cheaper. Consumers who lack knowledge on mites may not be aware about the effectiveness of the cleaning process in removing mites and other contaminants if they cleaned the nests themselves. Boiling the nests may not help very much. The mitecontaminated food can still elicit allergic reactions even though the food has been cooked (Erben et al., 1993; Wen et al., 2005; Tay et al., 2008). This is consistent with the fact that certain mite allergens (group 2 allergens) are heat resistant (Sanchez-Borges et al., 2005). Despite the denaturation and destruction of the allergens during the boiling process, the protein sequences of the allergens themselves may still be able to elicit allergic responses. Furthermore, for the preparation of EBN soup or drink, one may

use more than one nest, since the nests are quite small in size. If there is a threshold level for mites and mite allergens in ingested food, above which the consumer will develop allergic symptoms, then the abundance of mites/mite load in EBNs may be of great importance. EBNs with higher mite density will have a greater possibility of triggering allergy and anaphylaxis, compared with those with lower mite densities.

In North America, current standards permit up to 75 "insect fragments" per 50 g of grain (Sanchez-Borges *et al.*, 2005). However, concerns about the permissible level of mites and mite allergens in EBNs, or how much EBN can be consumed safely without triggering any untoward symptoms, remain unresolved. There is no such guideline in place either in Malaysia or other countries.

One of the measures to reduce the mite load in EBNs consumed by humans would be to address the sources of mites in the raw nests. There are a few possible sources of mites in raw EBNs. One would be the fauna and detritus found in the caves or swiftlet farms. Some mites are scavengers and feed on mosses, lichen, fungi, decaying leaves and wood, and other sources of detritus (Hughes, 1976). It is not unlikely that the nests of the swiftlets would also contain some of these food sources, therefore the mites may crawl into the nests and continue living there. Some mites are associated with insects, e.g. *Microlichus* sp. and *Myialges* sp. are associated with louse flies (Hippoboscidae) (Philips, 2000). Since swiftlets ingest insects, remnants of insects and possibly mites may be left in their mouths. When the swiftlets produce saliva to build their nests, the mite remnants may be incorporated into the nests.

Another source of mites is from the swiftlets themselves, since many species of mites have been associated with birds. Some mites are found in the respiratory system of birds e.g. mites from the families Ereynetidae (Boydaia sp. and Speleognathopsis sp.), Rhinonyssidae (Falconyssus sp., Ptilonyssus sp. and Tinaminyssus sp.) and Turbinoptidae (Schoutedenocoptes sp.) live in various levels of the respiratory system of falconiform birds (Philips, 2000; Proctor & Owens, 2000). Respiratory mites of the swiftlets may possibly be incorporated into the nests when the swiftlets build their nests with their saliva.

Some mites inhabit the feathers and skin of birds (Philips, 2000; Proctor & Owens, 2000), and are likely to get transferred to their nests. Feather mites are astigmatid mites from the superfamilies Analgoidea, Freyanoidea and Pterolichoidea that live on the feathers and skin of birds (Proctor, 2003). Examples of feather mites and their hosts include *Dubininia melopsittaci* on psittacine birds (Schmaschke *et al.*, 2002), *Pteroherpus garrulacis* on laughing trushes (Passeriformes: Timaliidae) (Mironov & Proctor, 2011), and *Laminalloptes* sp. on *Phaeton* sp. and *Fregata* sp. (Atyeo & Peterson, 1967).

Some mites are found not predominantly on the birds, but in the nesting material itself. These include mites which feed on the dermal detritus, fungi, algae, bacteria and other arthropods in the nest (Proctor & Owens, 2000; Stamp et al., 2002), and blood-feeding mites which only visit their host for short periods of time to feed but remain primarily nest-bound at other times (Proctor & Owens, 2000). One example of such mites is *Dermanyssus* gallinae, also called the red fowl mite or poultry red mite. Dermanyssus gallinae has a wide geographical distribution and low host specificity, having more than 30 avian hosts (Roy & Chauve, 2007). Dermanyssus *gallinae* has been reported to cause pruritic dermatitis when they leave abandoned bird nests, invade nearby human dwellings and bite humans (Cafeiro et al., 2013).

Besides the mites, fungal elements (hyphae and spores) and bacteria (rod and cocci) were observed on the surfaces of both raw and commercial EBNs. According to Standard and Industrial Research Institute of Malaysia (SIRIM) on Edible Bird Nest (EBN) Specification (MS 2334: 2010), the total plate count should be  $\leq 2.5 \times 10^6$ CFU/g, the coliforms should be  $\leq 1100 \text{ most}$ probable number (MPN)/g, Escherichia coli and Staphylococcus aureus should be  $\leq 100$ MPN/g, and no Salmonella species in all EBNs. The yeast and mold in the EBNs should be <10 CFU. The possible sources of fungi and bacteria in these nests may originate from the surroundings of the house farm

and the birds, and may be the contaminants introduced during the processing, storage and transportation of the EBNs.

Fungi are known to cause deterioration of food and its processed products via production of mycotoxins, which can affect their quality and safety. Mycotoxins are secondary metabolites of fungi and may include aflatoxins, ochratoxin A, fumonisins, trichothecenes and zearalenone (Pitt, 2000). Aflatoxins are produced by Aspergillus species in a variety of crops including peanuts, dried fruit, spices and maize (Caldas & Jardim, 2012). Aflatoxins are potent carcinogens or teratogens, and cause acute liver damage and cirrhosis. Ochratoxins A are produced by *Penicillium* species in cereal grains, grapes, wines and coffee beans, which cause kidney damage and urinary tract cancer. Fumonisins are produced by Fusarium species mainly found on maize and its products. Ingestion of fumonisins contaminated food may cause oesophageal cancer and neural tube defects. Trichothecenes and zearalenone are produced by Fusarium graminearum and other related species. Trichothecenes are highly immunosuppressive while zearalenone are with oestrogenic effects. The presence of fungal structures on the surface of the EBNs may affect their quality and safety. Further studies should be carried out to determine the mycotoxin and fungal loads in EBNs.

Apart from mycotoxins, inhalation of fungal spores and direct skin contact with fungal elements may lead to opportunistic infections especially in immunocompromised individuals and the development of allergic responses in others. This is of great concern to swiftlet farmers and public who stay nearby to the farm houses. Many species of fungi such as those from the genera of Alternaria, Fusarium, Cladosporium, Penicillium, Saccharomyces, Trichoderma have been associated with the symptoms of respiratory allergy (Kurup et al., 2000). Inhalation of spores of Aspergilus species causes pulmonary aspergillosis.

Bacteria can be beneficial or harmful to consumers. The beneficial bacteria could include but are not limited to *Lactobacillus* species, *Bifidobacterium bifidum*, *Enterococcus faecium* (de Roos & Katan, 2000). The majority of bacteria are harmful to humans except the normal flora. Bacteria possess nitrate reductase which catalyses reduction of nitrate to nitrite (Moir & Wood, 2001). Under the aerobic condition, the reduction of nitrate to nitrite by bacteria is inhibited and hence the nitrite level is higher in swiftlet ranches. Furthermore, bacteria are able to convert the ammonia derived from bird's faeces and urine into nitrate and nitrite (Hooper *et al.*, 1997). This may have led to the recent scare where a high level of nitrite was detected in those EBNs exported to China.

The commercial nests had guite a number of unidentified structures that were not seen in the raw nests, and were coated with a foreign substance, in comparison to the surface of the raw nests. These could be introduced during or after the nest processing cycle and may be due to addition of adulterants such as karaya gum. The adulterants (karaya gum, red seaweed and tremella fungus) may be the potential sources of allergens in the processed EBNs. Food quality control agencies should perform regular checks on the nest processing cycle, amount of additives added and determine whether the types and levels of additives added are safe for human consumption. The Standard and Industrial Research Institute of Malaysia (SIRIM) has initiated several guidelines and standards for EBNs from swiftlet ranching until packaging and storage. Among the enforced standards are the Good Animal Husbandry Practice: Edible Bird Nests Swiftlet Ranching and its Premises (MS 2273: 2012), Good Manufacturing Practice (GMP): For Processing Raw-unclean and Raw-clean Edible Bird Nests (EBN) (MS 2333: 2010) and Edible Bird Nest (EBN) Specification (MS 2334: 2010).

To date, no measures have been taken to determine or quantify the amount of mites in the commercial EBNs. No indicator has been put in place to prompt or alert the public especially those who are allergic to mites and other microbial allergens. A proposal on food allergen labelling and consumer protection could be introduced in order to reduce the number of mite or food-induced

anaphylactic cases. Various steps need to be taken in order to reduce the mite and microbial loads in the commercial EBNs. In order to decrease the levels of the contaminants such as mites, fungi, bacteria and feather strands, the stringency and effectiveness of the cleaning processes of the raw EBNs should be emphasised, and measures should also be taken to avoid contamination of the commercial EBNs during transportation and storage. Companies that sell commercial EBNs should take measures in enforcing the above. Prevention of mite and microbial contaminations can be achieved by storing commercial EBNs in proper storage conditions which are not optimum for the survival and growth of mite and microbial colonies. The recommended conditions to control mite population include not storing the food item for more than 6 months, and to keep the food in dry and cool places (storage temperature at 0-7°C and humidity of less than 12%) (Sanchez-Borges et al., 2005). An optimum storage condition should be determined to minimise microbial contamination. Further investigations should also be performed to identify the additives added to the EBNs and to determine if these additives are detrimental to the health of consumers.

In conclusion, structural analysis of the nests using scanning electron microscope revealed that mites, fungi, bacteria and feather strands were found in both raw and commercial nests. The commercial nests had quite a number of unidentified structures that were not seen in the raw nests, and were coated with a foreign substance, in comparison to the surface of the raw nests. These could be introduced during or after the nest processing cycle. Relevant food quality control agencies should perform regular checks on the nest processing cycle, amount of additives added and determine whether the types and levels of additives added are safe for human consumption. Various steps need to be taken in order to reduce the mite load in commercial EBNs also. To decrease the levels of these contaminants, the stringency and effectiveness of the cleaning processes of the raw EBNs should be emphasised, and measures should also be taken to avoid contamination of the commercial EBNs during transportation and storage.

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## REFERENCES

- Atyeo, W.T. & Peterson, P.C. (1967). The feather mite genus *Laminalloptes* (Proctophyllodidae: Alloptinae). *Kansas Entomology Society* **40**: 447-458.
- Cafiero, M.A., Camarda, A., Galante, D., Mancini, G., Circella, E., Cavaliere, N., Santagada, G., Caiazzo, M. & Lomuto, M. (2013). Chapter 26: Outbreaks of red mite (*Dermanyssus gallinae*) dermatitis in city-dwellers: an emerging urban epizoonosis. In: *Hypothesis in Clinical Medicine*. Nova Science Publisher, Inc., pp. 413-424.
- Caldas, E.D. & Jardim, A.N.O. (2012). Exposure to toxic chemicals in the diet: Is the Brazilian population at risk? Journal of Exposure Science and Environmental Epidemiology **22**(1): 1-15.
- Chao, P.L., Cui, Y.B., Wang, J., Yang, Q.G. & Tian, Y. (2003). Acaroid mite, intestinal and urinary acariasis. World Journal of Gastroenterology 9(4): 874-877.
- Colloff, M.J. (2009a). Chapter 7: Dust mite allergens. In: *Dust mites*. 1<sup>st</sup> edition. Australia: CSIRO Publishing, pp. 273-328.
- Colloff, M.J. (2009b). Chapter 8: Allergy and epidemiology. In: *Dust mites*. 1<sup>st</sup> edition. Australia: CSIRO Publishing, pp. 329-338.
- de Roos, N.M. & Katan, M.B. (2000). Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. American Journal of Clinical Nutrition **71**(2): 405-411.
- Erben, A.M., Rodribuez, J.L., McCullough, J. & Ownby, J.R. (1993). Anaphylaxis after ingestion of beignets contaminated with Dermatophagoides farinae. The Journal of Allergy and Clinical Immunology 92: 846-849.

- Goh, D.L.M., Chew, F.T., Chua, K.Y. & Chay, O.M. (2000). Edible "bird's nest"-induced anaphylaxis: an under-recognised entity? *The Journal of Pediatric* 137(2): 277-279.
- Goh, D.L.M., Lau, Y.N., Chew, F.T., Shek, L.P.C. & Lee, B.W. (1999). Pattern of foodinduced anaphylaxis in children of an Asian community. *Allergy* 54: 78-92.
- Goh, L.M., Chua, K.Y., Chew, F.T., Seow, T.K., Ou, K.L., Yi, F.C. & Lee, B.W. (2001). Immunochemical characterisation of edible bird's nest allergens. *The Journal* of Allergy and Clinical Immunology **107**(6): 1082-1087.
- Hon, K.L.E., Leung, T.F., Hung, C.W.E., Cheung, K.L. & Leung, A.K.C. (2009). Ingestion associated adverse events necessitating pediatric ICU admissions. *Indian Journal of Pediatrics* **76**(3): 283-286.
- Hon, K.L.E., Leung, T.F., Kam, W.Y.C., Lam, M.C.A., Fok, T.F. & Ng, P.C. (2006). Dietary restriction and supplementation in children with atopic eczema. *Clinical* and Experimental Dermatology **31**: 187-191.
- Hooper, A.B., Vannelli, T., Bergmann, D.J. & Arciero, D.M. (1997). Enzymology of the oxidation of ammonia to nitrite by bacteria. Antonie van Leeuwenhoek 71(1-2): 59-67.
- Hughes, A.M. (1976). The subclass Acari. In: *The mites of stored food and houses*. 2<sup>nd</sup> edition. London: Her Majesty's Stationery Office, pp. 1-5.
- Kemp, A., Chiang, W.C., Gerez, I., Goh, A., Liew, W.K., Shek, L., van Bever, H.P. & Lee, B.W. (2010). Childhood food allergy: a Singaporean perspective. *Annals Academy of Medicine Singapore* **39**: 404-411.
- Kurup, V.P., Shen, H.D. & Banerjee, B. (2000). Respiratory fungal allergy. *Microbes Infection* 2(9): 1101-1110.
- Lim, C.K. (2011). Opportunity and sustainability of swiftlet farming in Malaysia. *ICOTOS* 1-16.
- Ma, F. & Liu, D. (2012). Sketch of the edible bird's nest and its important bioactivities. *Food Research International* **48**(2): 559-567.

- Marcone, M.F. (2005). Characterisation of the edible bird's nest the "caviar of the East". *Food Research International* **38**(10): 1125-1134.
- Matsumoto, T., Goto, Y. & Miike, T. (2001). Anaphylaxis to mite-contaminated flour. *Allergy* **56**: 247-265.
- Matsumoto, T. & Satoh, A. (2004). The occurrence of mite-containing wheat flour. *Pediatric Allergy Immunology* **15**(5): 469-471.
- Mironov, S.V. & Proctor, H.C. (2011). Four new feather mite species of the family Pteronyssidae (Astigmata: Analgoidea) from laughing-thrushes (Passeriformes: Timaliidae) in China. *Acarina* **19**(1): 35-51.
- Moir, J.W.B. & Wood, N.J. (2001). Nitrate and nitrite transport in bacteria. *Cellular and Molecular Life Sciences: CMLS* **58**(2): 215-224.
- Nadchatram, M. (2005). House dust mites, our intimate associates. *Tropical Biomedicine* **22**(1): 23-37.
- Philips, J.R. (2000). A review and checklist of the parasitic mites (Acarina) of the falconiformes and strigiformes. *Journal* of Raptor Research **34**(3): 210-231.
- Pitt, J.I. (2000). Toxigenic fungi and mycotoxins. British Medical Bulletin 56(1): 184-192.
- Proctor, H. & Owens, I. (2000). Mites and birds: diversity, parasitism and coevolution. *Trends in Ecology and Evolution* **15**(1): 358-364.
- Proctor, H.C. (2003). Feather mites (Acari: Astigmata): ecology, behaviour and evolution. *Annual Review of Entomology* **48**: 185-209.
- Roy, L. & Chauve, C.M. (2007). Historical review of the genus *Dermanyssus Duges*, 1834 (Acari: Mesostigamata). *Parasite* 14: 87-100.
- Sanchez-Borges, M., Capriles-Hulet, A., Fernandez-Caldas, E., Suarez-Chacon, R., Carabello, F., Castillo, S. & Sotillo, E. (1997). Mite contaminated foods as a cause of anaphylaxis. *The Journal of Allergy and Clinical Immunology* **99**: 738-743.

- Sanchez-Borges, M., Fernandez-Caldas, E., Capriles-Hulett, A. & Caballero-Fonseca, F. (2012). Mite-induced inflammation: more than allergy. *Allergy and Rhinology* **3**: e25-29.
- Sanchez-Borges, M., Suarez-Chacon, R., Capriles-Hulet, A. & Carabello-Fonseca, F. (2005). An update on oral anaphylaxis from mite ingestion. *Annals of Allergy*, *Asthma and Immunology* **94**: 216-220.
- Schmaschke, R., Schone, R., Sachse, M. & Eulenberger, K. (2002). Feather mites of Psittacidae. European association of Zoo and Wildlife Veterinatians (EZAWV) 4<sup>th</sup> scientific meeting, joint with the annual meeting of the European Wildlife Disease Association (EWDA). Heidelbarg, Germany.
- Stamp, R.K., Brunton, D.H. & Walter, B. (2002). Artificial nest box use by the North Island saddleback: effects of nest box design and mite infestations on nest site selection and reproductive success. *New Zealand Journal of Zoology* 29(4): 285-292.
- Stoehr, A.M., Nolan, P.M., Hill, G.E. & McGraw, K.J. (2000). Nest mites (*Pellonyssus reedi*) and the reproductive biology of the house finch (*Carpodacus mexicanus*). *Canadian Journal of Zoology* **78**: 2126-2133.

- Tay, S.Y., Tham, E., Yeo, C.T., Yi, F.C., Chen, J., Cheong, N., Chua, K.Y. & Lee, B.W. (2008).
  Anaphylaxis following the ingestion of flour contaminated by house dust mites a report of two cases from Singapore. *Asian Pacific Journal of Allergy and Immunology* 26: 165-170.
- Thong, B.Y.H., Cheng, Y.K., Leong, K.P., Tang, C.Y. & Chng, H.H. (2005). Anaphylaxis in adults referred to a clinical immunology/ allergy centre in Singapore. *Singapore Medical Journal* **46**(10): 529-534.
- Thong, B.Y.H., Cheng, Y.K., Leong, K.P., Tang, C.Y. & Chng, H.H. (2007). Immediate food hypersensitivity among adults attending a clinical immunology/allergy centre in Singapore. *Singapore Medical Journal* 48(3): 236-240.
- Wen, D.C., Shyur, S.D., Ho, C.M., Chiang, Y.C., Huang, L.H., Lin, M.T., Yang, H.C. & Liang, P.H. (2005). Systemic anaphylaxis after the ingestion of pancake contaminated with the storage mite *Blomia freemani*. *Annals of Allergy, Asthma and Immunology* **95**: 612-614.
- Zhang, J. (2012). Edible bird's nest banned to bring in China. http://english.cntv.cn/ 20120227/116881.shtml. Source: Xinhua.