Surface Morphology Study on Unclean, Commercial and Bromelain treated Edible Bird Nest (EBN) using Scanning Electron Microscope

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Abstract. Edible Bird Nest (EBN) is a bird made product and its one of the most expensive animal products consumed by human nowadays due to high nutritional values and medicinal properties. Market processed EBN found the presence of mites, fungal spores and feather strands using scanning electron microscope (SEM). Mite was the common cause of anaphylaxis. Indeed, mite are heat resistant and thus can elicit allergic reaction even the food has been cooked. Therefore, the enzyme technology is applied in the current study on EBN to remove most of the pathogen. The results obtained in this study were attributable to the fact that enzyme treatment EBN is added value product and safer for human consumption as compared to that of the market processed EBN.

1. Introduction
Edible Bird Nest (EBN) is consumed by many Chinese as a symbol of wealth, power, prestige and as well as being used medicinally in traditional Chinese medicine. Many Chinese people used it in their cuisine and this delicacy is valued for its high nutritional content and medicinal benefits [1]. Thus, swiftlet farming has expanded rapidly in Southeast Asia [2] due to increased global demand for EBNs especially China and Malaysia is one of the largest exporter of EBNs to China. Indeed, the raw nests required to go through the process of soaking, cleaning, bleaching, moulding and packaging before they are sold as cleaned commercial EBN [3]. The cleaning process of EBN in industries mainly only focus on removal of feather strands as it can be observed by the naked eye of the consumer. However, the commercial cleaned EBN may contain some pathogen which can cause illness to the consumer. Pathogen normally can only be observed under high magnification microscope such as scanning electron microscope.

Mites, fungi, bacteria and feather strands were normally founded on the structural analysis of raw and commercial EBN using scanning electron microscope [4].

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Furthermore, there is no documentation to date has reported the concerns on safe consumption of EBN. However, there are some report on allergic symptoms and food induced anaphylaxis after consumption of EBN. EBN was the common cause of anaphylaxis, surpassing other well-defined food allergens, such as egg cow’s milk, peanut or crustacean seafood in children [5]. Exposure to a level over the individual tolerable or threshold level of allergen concentration will increase the chance of development of allergy [4].

Nowadays, technology of enzymes, especially bromelain is widely used in many fields such as for leather and textile industries hair removal, wool, skin softening and detergent formulation [6]. In fact, Bromelain is the type of proteolytic enzyme that serves to break down protein molecules. The sulfhydryl group (cysteine and histidine) on the active site of the Bromelain which can be used to break the bonds of fungal and bacterial protein [7]. Consequently, it is expected that could inhibit the growth of fungal and bacteria and remove mites from EBN. Therefore, this study was designed to examine whether the enzyme cleaning method is efficiently removed the mites, fungus and bacteria from EBN.

2. Materials and Methods

2.1. EBN sample collection
Raw unclean and commercial EBN were purchased from Changloon, Kedah. The EBN was stored in an airtight container and kept in room temperature.

2.2. Enzyme extract
The skin of the pineapple was removed with a knife and cut into small cube which including the stem. After that, the pineapple cubes being blended and then filtered on cheesecloth to remove remaining pulp. The extract was centrifuged at 8,000 rpm for 20 min in centrifuge to get a pure and clear extract. The extract was mixed with pH 7 phosphate buffer at the ratio of 1:1 before stored at 4 °C to retain the enzymatic activity of the Bromelain.

2.3. Purification of Bromelain by three phases partitioning (TPP)
Three phases partitioning (TPP) precipitation is one of the new methods which is effective in the separation and purification of enzymes. A defined amount of ammonium sulfate and tertiary butanol were added to the crude enzyme and made up to 50 mL. The mixture was then undergoing centrifugation at 5,000 rpm for 20 min at 4 °C. The precipitate was retrieved and dissolved in deionized water contained vial. The solution was dialyzed for eight times at 4 °C using 50 mM phosphate buffer at pH7. The solution was then freeze dry at - 80 °C to remove excess moisture.

2.4. Bio enzyme cleaning process of EBN
Firstly, the EBN was soaked in the 50g/l of purified Bromelain solution for 15 min. Then, the EBN was placed in centrifuge with 5,000 rpm for 10 min to separate the impurities and EBN. The cleaned EBN were rearranged and dried under room temperature. The dried EBN was stored in an airtight container before structural analysis.

2.5. Structural analysis of EBN
The surface structures and morphology of the dried enzyme treated EBN, commercial EBN and unclean EBN samples were observed using scanning electron microscope (SEM, JEOL JSM-6460LA, Tokyo Japan) operating at an accelerating voltage of 10 kV. The EBN samples were first crushed into small fragments and placed on the stage with carbon tape before coated with titanium particles. The structures of EBN and contaminants were then examined and recorded.
3. Result and Discussion
The changes in the surface morphology of the unclean EBN, enzyme treated EBN and Commercial EBN samples were analysed using SEM in order to determine the effectiveness of the enzyme cleaning method. Figure 1 shows the SEM images of unclean EBN while Figure 2 and Figure 3 illustrate the SEM images of commercial and enzyme treated EBN, respectively. Under the SEM, few structures were seen on the surface of the raw nest including mites (Figures 1c, 2b), yeast or fungal spores (Figures. 1d, 2d, 3b, 3c, 3d) and feather strands (Figures. 1b, 2c).

In fact, raw EBN is sold in the market is about of the half of the price of commercial EBN. Nevertheless, raw EBN contains more contaminants as compared with commercial EBN. The raw EBN almost fully covered by bacteria as shown in Figure 1a. The feather strand of swiftlet is all over the EBN while mites normally embedded in or on the surface of EBN. This is because tropical countries are more favourable for mites to survive and build up huge population [8]. The consumer who lacks of knowledge on mites may clean the raw EBN in inadequate ways in removing mites and other contaminants. Certain mite allergens are heat resistant as a result of the protein sequences of the allergen still able to trigger the allergic responses even after the denaturation or destruction during the boiling process. Thusly the mite contaminated food still can elicit allergic reactions regardless of the food has been cooked [9].

Shockingly, even after cleaning process by industry, the mites and plume strands have been yet found in the commercial EBN. The structural analysis of commercial EBN uncovered the presence of mites and fungal spores (Figure 2b, 2d). Commercial EBN contain lesser contaminants because of the cleaning procedure applied could partially evacuate the contaminants. The cleaning procedure in the industry normally soak the EBN in a huge amount of water to soften it before the removal of feather utilizing tweezers by skilled labour. Indeed, even with the cleaning procedure, the mites and strands of feathers were still can be found on the surface of the commercial nest (Figure 2b, 2c).

Lastly, mites and plume strand are not visible in the EBN after the enzyme cleaning process (Figure 3). The EBN contain lesser fungal spores as compared to that of the raw and commercial EBN (Figure 1d, 2d, 3d). This is due to bromelain can break down the bond of fungus protein and remove the mites. Eventually remove most of the visible fungus and mites in the EBN. Therefore, the enzyme technology is capable to apply on EBN cleaning process to remove the allergen source. As result, the enzyme cleaned EBN is safer for human consumption.
Figure 1. SEM micrograph of raw EBN at magnification of (a) 33x, (b) 100x, (c) 300x, (d) 1000x

Figure 2. SEM micrograph of commercial EBN at magnification of (a) 33x, (b) 200x, (c) 200x, (d) 1000x
4. Conclusion
In conclusion, surface morphology study of the EBN using scanning electron microscope revealed that feather strand, mite, fungi and bacteria were mainly found in raw and commercial EBN. Thus, to minimize the mite and microbial growth in commercial processed EBN, a new bio-based enzyme cleaning method is developed in the current study. The enzyme cleaned EBN was having the lowest feather strand, fungal and mites as compared to that of the raw and commercial EBN. The results may be attributable to the fact that enzyme treatment is better and could result in a safer EBN for human consumption.

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