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Improving Drying Characteristics and Quality Attributes of Edible Bird's Nest (EBN) Processed under Intermittent IR-UVC Assisted Drying

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ABSTRACT

The quality of Malaysian edible bird's nest (EBN) is still regarded inferior due to issues related to nitrite content. In August 2011, the Chinese government imposed a ban on EBN products imported from Malaysia, due to the high level of detectable nitrite (NO_2^-). This nitrite contamination issue has made a great impact to the edible bird's industry whereby many EBN farmers, traders and exporters are severely affected. In 2012, the China-Malaysia negotiations agreed on the use of radio frequency identification (RFID) tags on every individual package of Malaysian EBN and set the allowable nitrite limit to be less than 30 ppm, in order to allow exportation of Malaysian EBN to China.

The processing methods in EBN industry still remain crude and lack of any form of technology advancement. The purpose of this study was to investigate infrared (IR) coupled with ultraviolet C (UVC) assisted intermittent drying as a mean of EBN processing in order to preserve its nutraceutical properties, ensure nitrite content less than 30 ppm and enhance the commercial value of the dried EBN products. In the present study, EBN were dried by IR assisted drying at low temperature (25°C, 26.7% RH and intermittency =1.00), UVC assisted drying at low temperature (25°C, 26.7% RH and intermittency=1.00) and IR-UVC assisted drying (25°C-40°C, 16.5%-26.7% RH and intermittency=0.20-1.00), respectively. Both engineering properties (drying kinetics, effective moisture diffusivity and effective thermal diffusivity) and quality properties (colour, nitrite content, moisture reabsorption ability, shrinkage, sialic acid retention, antioxidant retention and storage stability) of all dried products were assessed and evaluated. The comparisons were made against samples from fan assisted drying and hot air drying.

The results revealed that intermittent infrared (IR) coupled with ultraviolet C (UVC) assisted drying at temperature of 40°C (IR-UVC40) with intermittency, α of 0.20-1.00 is the best drying method among all experimental treatment because it allowed faster effective drying at mild temperature as compared to other drying methods. Drying at the relatively low temperature had also produced dried product with premium quality and better storage stability. The total colour change of EBN sample under intermittent infrared (IR) coupled with ultraviolet C (UVC) assisted drying at both temperature of 40°C and 25°C, intermittency, α = 0.20 is not significant changed ($p<0.05$) as compared to fan assisted dried and hot air dried EBN samples. Moreover, the nitrite content of infrared (IR) coupled with ultraviolet C (UVC) assisted dried samples reduced significantly *($p<0.05$)* as compared to infrared (IR) or ultraviolet C (UVC) assisted dried samples.

Optimization on three drying variables such as intermittent duration (X_1), intermittent ratio (X_2) and intermittent cycle (X_3) was performed by using response surface methodology (RSM) with the aims to determine the drying profile, in order to avoid excessive long drying period, and also to improve the product quality as well as storage stability. The quadratic response surface demonstrated that there was an optimum retention of sialic acid (89.97 mg sialic acid/ g dry weight) and antioxidant contents (88.65 ± 0.25 mg TEAC/g dw) at intermittent ratio between 0.40-0.44 with the optimum drying duration. Therefore, the optimal intermittent drying parameters were determined at intermittent duration (X_1) of 415 min, intermittent ratio (X_2) of 0.40 and intermittent cycle (X_3) of 3. After six months of storage, the increment of nitrite content in all dried EBN samples was not significantly different ($p > 0.05$) and remained less than 30 ppm, except for the fan assisted dried samples. Therefore, the potential of infrared (IR) coupled with ultraviolet C (IR-UVC) assisted drying has been proven through this work and able to improve the quality of Malaysian EBN.

PUBLICATIONS

Part of the studies in this dissertation have been submitted for publications.

Book Chapter

i) **Gan, S.H.**, Law, C.L., and Ong, S.P., 2015. Review on Challenges and Future Drying Trend in Edible Bird's Nest Industry. In *Processing of Foods, Vegetables and Fruits: Recent Advances,* eds. C.L. Hii, S.V. Jangam, S.P. Ong, P.L. Show, A.S. Mujumdar, pp. 45-60.

International Journals

i) **Gan, S.H.**, Ong, S.P., Chin, N.L., and Law, C.L., 2016. Colour Change, Nitrite Content and Rehydration Capacity of Edible Bird's Nest by Advanced Drying Method. *Drying Technology: An International Journal,* 34(11), pp.1330-1342. Available at http://dx.doi.org/1001080/07373937.2015.1106552.

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iv) **Gan, S.H.**, Ong, S.P., Chin, N.L., and Law, C.L., 2016. Retention of Sialic Acid Content in Malaysian Edible Bird's Nest by Heat Pump Drying. *Malaysian Journal of Veterinary Research,* 8(1).

Conference Papers

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ii) **Gan, S.H.**, Law, C.L., and Ong, S.P., 2015. Retention of Sialic Acid Content in Edible Bird's Nest by Heat Pump Drying. In *The 8th Asia-Pacific Drying Conference (ADC 2015)*, Kuala Lumpur, Malaysia, August 10-12, 2015, pp. 92-99.

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iv) **Gan, S.H.**, Law, C.L., and Ong, S.P., 2015. Improving Edible Bird's Nest Quality and Energy Efficiency by Heat Pump Drying. In *The 8th Asia-Pacific Drying Conference (ADC 2015),* Kuala Lumpur, Malaysia, August 10-12, 2015, pp. 109-116.

v) **Gan, S.H.**, Law, C.L., and Ong, S.P., 2015. Kinetic Modelling of Sialic Acid and Antioxidant Degradation during Low Temperature Drying of Edible Bird's Nest. In *The 20th International Drying Symposium (IDS 2016),* Gifu, Japan, August 8-11, 2016.

vi) **Gan, S.H.**, Law, C.L., and Ong, S.P., 2015. Study on Retention of Metabolites Composition in Misai Kucing *(Orthosiphon Stamineus)* by Solar Assisted Heat Pump Drying. In *The 20th International Drying Symposium (IDS 2016),* Gifu, Japan, August 8-11, 2016.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF NOTATIONS AND ABBREVIATIONS

Notations

a^*	CIE redness value
a_W	Water activity
b	Correlation parameter
b^*	CIE yellowness value
C	Nitrite or nitrate in samples for measurement (mg/L)
C_p	Heat capacity (J/kg.K)
d_p	Diameter of particle (m)
D_{ab}	Diffusivity for air-water mass transfer (m^2/s)
D_o	Arrhenius constant (m^2/s)
D_{eff}	Effective diffusivity (m^2/s)
ΔE	CIE colour difference
E_a	Activation energy (kJ/mol)
EMC	Equilibrium moisture content (g H_2O/g dry solid)
f	Dilution factor of sample solution
h_m	Mass transfer coefficient (m/s)
h_t	Heat transfer coefficient ($W/m^2.K$)
k_1, k_2, k_3, k_4	Drying kinetic constants (1/s)
k	Rate constant
K	Thermal conductivity (W/m.K)
L	Length (m)
L^*	CIE lightness value
m_{rh}	Mass of rehydrated sample (g)
m_{dh}	Mass of dry sample taken for rehydration testing (g)
M_a	Molecular weight of water vapour (g/mol)
M_b	Molecular weight of air (g/mol)
M_{in}	Moisture content (% dry basis) of sample before drying
M_{dh}	Moisture content (% dry basis) of the dry sample
n_1, n_2, n_3, n_4	Correlation parameters
N_{coded}	Coded value
N_i	Corresponding actual value
N_o	Actual value in the centre of the domain
ΔN	Increment of N_i corresponding to a variation of 1 unit of N
P_T	Total pressure (atm)

r	Radius (m)
R	Universal gas constant (J/mol.K)
S	Shrinkage
t	Time (s)
t_d	Thickness of the sample after drying (m)
t_o	Thickness of the raw sample of edible bird's nest (m)
t_{drying}	Drying period (hr)
$t_{tempering}$	Tempering period (hr)
T	Absolute temperature (K)
T_{ON}	"On" period of the dryer
T_{OFF}	"Off" period of the dryer
V	Volume of sample solution (mL)
W	Weight fraction
W_o	Initial weight of product (g)
W_d	Weight of dry matter in product (g)
$X_{nitrite}$	Nitrite or nitrate content in sample (ppm)
X_i	Moisture content at time *i* (g H_2O/g dry solid)
X_e	Equilibrium moisture content (g H_2O/g dry solid)
X_o	Initial moisture content (g H_2O/g dry solid)

Greek symbols

α	Intermittency
$\alpha_{thermal}$	Thermal diffusivity (m^2/s)
ρ	Density(kg/m^3)
μ	Viscosity (Pa.s)

Abbreviations

ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid assay)
DHC	Dry basis holding capacity index
DPPH	1,1-diphenyl-2-pycrylhydrazyl free radical scavenging
EBN	Edible bird's nest
EGF	Epidermal growth factor
FAN27	Fan drying
FIR	Far-infrared
FRAP	Ferric reducing antioxidant power assay
HA	Hot air drying
HP	Heat pump drying
LT	Low temperature drying
IR25	Infrared (IR) assisted drying at 25°C
UVC25	Ultraviolet C (UVC) assisted drying at 25°C
IR-UVC25	IR-UVC assisted drying at 25°C
IR-UVC40	IR-UVC assisted drying at 40°C
MR	Moisture ratio
MIR	Middle-infrared
NIR	Near-infrared
RA	Moisture reabsorption ability
TAC	Total antioxidant capacity
TEAC	Trolox equivalents antioxidant capacity
WAC	Water absorption capacity index

LIST OF APPENDICES

CHAPTER 1 INTRODUCTION

1.1 Background of Study

Edible bird's nest (EBN) (also known in Chinese as "Yan Wo", Indonesian as "Sarang Walet" and Japanese as "Ensu") refers to the nest produced by the several swiftlet species in the genus of *Aerodramus fuciphogus* and *Aerodramus maximus* (Koon, 2000). The nests are constructed from the saliva of swiftlet, which has been secreted from the pair of sublingual salivary gland of swiftlets during nesting and breeding season (Koon, 2000). EBN are graded according to the location where the nests are built, edibility, external features, colour, quality and cleanliness. It can be sorted into cave EBN and farm EBN. The end products of EBN can be whole piece, narrow piece and biscuits.

With the rapid increasing awareness of consumers to health and beauty, EBN is considered as a highly esteemed food tonic. It is generally believed by the Chinese community that EBN has a high medicinal value. Number of claimed benefits of consuming EBN soup have been reported, which includes dissolving phlegm, relieving gastric troubles, aiding renal functions, raising libido, enhancing complexion, alleviating asthma, suppressing cough, strengthening immune system, speeding recovery illness and surgery, as well as increasing energy and metabolism (Konga et al., 1987; Ma and Liu, 2012). The recent researches have also shown that the extracts from EBN have a significant effect in inhibiting infection of influenza and avoiding bone loss (Ma and Liu, 2012).

Malaysia is currently ranked the second largest producer and exporter for EBN, which include both raw and processed EBN with RM 1.5 billion in sales. China is the largest importer for EBN with 82 % of the annual global sales, followed by South East Asia (13 %), United States and Canada (5 %). In 2007, annual sales of Malaysia EBN in the global market was about RM 10.2 billion. In 2009, the

price of cleaned and dried EBN was around RM 9,000 per kg in China retail market (Bird's Nests Traders Association of Malaysian, 2015; FFTC Agricultural Policy Platform, 2015; Malaysian Edible Farming Industry Report, 2007). Despite the popularity of EBN as a health supplement, it is challenging to ensure the quality of EBN as it is vulnerable to processing conditions and tends to degrade easily.

In August 2011, the Chinese government imposed a ban on EBN products imported from Malaysia, due to the high level of detected nitrite (NO_2^-) (AQSIQ, 2011; Food Quality News, 2011; Xinhuanet, 2012). Two major issues concerning the processing of EBN are high content of nitrite and severe colour change in cleaned dehydrated EBN due to improper handling and processing of the EBN. This has greatly affected the price of Malaysia EBN products in the global market. In end of the year 2011, the wholesale price of cleaned and dried EBN were dropped from RM 6,500 per kilogram to RM 1,500 per kilogram (Bird's Nests Traders Association of Malaysian, 2015; TheStar, 2015). The contamination issue has made a great impact to the EBN industry as well as many EBN farmers, traders and exporters are affected. This issue has also caused a great impact to our nation's EBN export (TheStar, 2015).

In 2012, the China-Malaysia negotiations stipulated the use of radio frequency identification (RFID) tags on every individual packages of Malaysian edible bird's nests and sets the allowable nitrite limit to be less than 30 ppm, in order to allow the export of Malaysia EBN to China (Bird's Nests Traders Association of Malaysian, 2009; AQSIQ, 2011). In 2015, the wholesale price of cleaned and dried EBN were slightly rebounding to RM 4,000 – RM 5,000 per kg, and retail price can be 2.5–8 times higher (Bird's Nests Traders Association of Malaysian, 2015; TheStar, 2015). Malaysian government has recognized the economic

potential of EBN industry and expected to contribute more than RM 5.2 billion to the Gross National Income (GNI) in 2020.

Hence, the government has established a comprehensive master plan for the development of EBN industry, such as introduced Agriculture National Key Economic Areas (NKEA) stated in Economic Transformation Programme 2011 and implemented Malaysian Standard of Edible Bird's Nest (MS 2334: 2011). The standard was established to regulate and monitor the quality of Malaysia EBN products for domestic and international markets. By building on Malaysia's status as a recognised supplier and resolving key challenges within the industry, Malaysia aims to produce 860 metric tonnes of EBN (capture 40% of the global market) by 2020 (Bird's Nests Traders Association of Malaysian, 2015). Swiftlet farming industry is a new emerging industry in Malaysia and it has achieved its critical mass. At year 2013, there are more than 3,000 commercial bird houses from emptied buildings such as old factories, old shops and dedicated bird's houses in Malaysia (FFTC Agricultural Policy Platform, 2015).

Nevertheless, the high level of nitrite found in EBN has raised public concern, casting doubt whether these EBNs are truly "edible". Nitrite has been used as a food preservative and antibotulinal agent in the food processing industry to protect against microorganisms growth. However, the nitrite level is strictly controlled to prevent food toxicity because nitrite can react with secondary amines in food products or in the digestive system to form nitrosoamines, a class of carcinogenic compounds (Department of Standard Malaysia, 2011). Also, nitrate can readily be converted into nitrite by microbial reduction. Many EBN manufacturers also found that nitrite and nitrate content in dried EBN products increased significantly during transportation of EBN from Malaysia to China mainland (Department of Standard Malaysia, 2011).

Postharvest processing of agricultural, herbal, food products and bio-materials typically requires a drying process to remove excess moisture or water content. This is required in order to lower the water activity to a level that is safe for storage and retards the growth of moulds and microorganisms (Fabiano et al., 2010; Mujumdar and Law, 2010). Likewise, in the swiftlet EBN processing industry, drying is required to reduce the water activity for the aforementioned purposes. EBN industry commonly uses air blowing machine such as fan or hot air drying system to dry EBN. Utilization of different drying techniques can affect the nitrite content, colour, shape, nutrients and flavour of the dried edible bird's nest (Banyan Bird Nest, 2010; Blissful Bird Nest, 2011; Malaysia Hydroponis, 2013).

After cleaning and drying using the conventional drying methods such as hot air drying or atmospheric air drying (i.e. fan assisted drying) in the prolonged period of time, the bird nest turns yellowish which is undesirable to consumers. This problem has been long existed in the industry. Operators in the EBN industry normally overcome the problem by applying bleaching agents which are chemical based and hazardous to health. The severe qualities issue such as browning (causing yellowish bird nest) and brittleness of EBN are mainly due to improper processing such as high temperature and long duration processing (GuangZhou News, 2011; SinChew News, 2015; ZheJiang News, 2011).

In terms of consumers' acceptability of EBN, colour and texture play very important roles in appearance of EBN. Colour is perceived as part of the total appearance, while texture is the visual recognition and assessment of the surface and subsurface properties of the EBN. The first quality judgement made by a consumer on EBN at point of sale is on its appearance. Sensory analyses of EBN (colour, taste, odour and texture) are used for the quality control throughout EBN processing. Colour is, perhaps the most important appearance attribute because

abnormal colours, especially those associated with deterioration in eating quality or with spoilage, cause the product to be rejected by the consumer.

Moreover, sialic acid content is established as unique indicator for the grades of real EBN. Sialic acid and antioxidant contents in EBN also affects the decision making of the consumer when purchasing EBN. The traditional processing method such as fan assisted drying and hot air drying are the most common methods that are applied in the EBN processing. These methods use natural air and supplies heat from heating elements to elevate the temperature of the drying medium. Typical drying time of EBN using this method is 4-12 hours depending on the operating temperature. High operating temperature (e.g. 80^{o}C) gives shorter processing time but severe colour change. In contrary, lower operating temperature (e.g. 40^{o}C) prolongs processing time thus results in lower retention of heat-labile bio-active ingredients such as sialic acid and antioxidant as well as significant colour change due to long duration of chemical reactions on the bio-active ingredients (Konga et al., 1987; Law et al., 2008).

Hence, technological advancement in the EBN processing is needed, particularly in the drying process of EBN. Infrared (IR) drying has become an emerging technique in the food industries because it is known as a drying technique that allows a high rate of water evaporation with minimum quality losses, such as colour changes, shrinkage, surface hardening, sample deformation, loss of aroma and ascorbic acid degradation (Riadh et al., 2015). IR drying also can be used to inactivate bacteria, spores, yeast and mould in both liquid and solid foods (Riadh et al., 2015). A number of foodstuff drying trials using IR dryer have been conducted in recent years, such as barley (Afzal et al., 1999), squid fillets (Deng et al., 2014), peanut (Rim et al., 2005), blueberry (Shi et al., 2008) and potato chips (Supmoon and Noomhorm, 2013).

Although IR drying is known to be promising, it also has the limitations in its penetrating power and not applicable for all drying systems. As a result, combination of electromagnetic radiation, such as ultraviolet C (UVC) irradiation, depending on the specific process, is usually applied to ensure better drying efficiency and better product quality (Riadh et al., 2015). Although available research has proved IR drying can improve the quality of heat sensitive high value foods like herbs and UVC can kill bacteria, but such findings on comparing the drying kinetics and product quality of EBN under low temperature drying with IR and UVC treatment have not been reported and documented to date.

1.2 Problem Statement

Malaysia edible bird's nest (EBN) industry has faced a crisis in terms of its exports recently. In 2009, the price of cleaned and dried EBN was around RM 9,000 per kg in China retail market (Bird's Nests Traders Association of Malaysian, 2015; FFTC Agricultural Policy Platform, 2015; Malaysian Edible Farming Industry Report, 2007). In August 2011, China as the main export destination banned Malaysia EBN due to the high level of nitrite of 200 ppm. This import restriction has caused accumulation of EBN in the country and a 77% decline in market value of EBN. As a result, EBN price plunged from RM 6,500 per kilogram to only RM 1,500 per kilogram (Borneo Post Online, 2012). To overcome this issue, there is an urgent need in research to look into possible source of nitrite and ways to reduce the nitrite content and enhance the EBN quality in term of colour, texture, flavour and bioactive ingredients retention via processing.

In this regard, drying is a crucial process step which requires further research. Moreover, the heat treatment history has great impact on the stability of dried EBN during storage. It was reported that the nitrite content of dried EBN had increased gradually during the storage and transportation period due to its high water activity (Borneo Post Online, 2012; Malaysian Edible Farming Industry

Report, 2007). The processing methods of EBN still remain crude and lack in technological advancements. Although many efforts have been made to investigate the drying characteristic on different types of food products, however, very little information is available on the effect of different drying methods on EBN, especially in terms of the EBN quality (Gan et al., 2015). Hot air drying is usually carried out at high air temperature (>60°C) and this could reduce sialic acid and antioxidant content in EBN which are beneficial to human health. The benefits of EBN as well as its sialic acid and antioxidative effects have been extensively reported in published literature (Gan et al., 2015; Guo et al., 2006; Ma and Liu, 2012; Yang et al., 2013). Therefore, the use of low temperature drying is conducive for drying EBN. It has been reported that low temperature drying could improve the colour attributes and texture of dried product, and also able to preserve the heat sensitive bioactive compounds (Chin and Law, 2010; Chong et al., 2008; Chong and Law, 2010; Ong and Law, 2011).

In order to maintain high quality dried EBN products with nitrite content less than 30 ppm, advancement in drying technology is needed such as combination of infrared (IR) and ultraviolet C (UVC) under low operating temperature and low relative humidity. This advanced drying technology could be a promising method in EBN processing because of its advantages such as better drying efficiency, shorter drying time, high quality dried products, better energy savings, intermittent energy source, easy control of process parameters, uniform temperature distribution, and clean operational environment, as well as space savings (Riadh et al., 2015).

Numerous research works have been carried out to study drying kinetics and product quality of foodstuffs by IR assisted drying and UVC assisted drying. Today’s consumer expectation for better quality, safety and nutritional value in EBN drives research and improvement of EBN drying technologies. Up to date,

there is no report on comparing the drying kinetics and product quality of EBN by IR-UVC assisted drying in the literatures. Hence, this study aimed to explore the drying kinetics and product quality of EBN by IR-UVC assisted drying.

1.3 Research Objectives

The main objectives of this research were:

- To evaluate the transport properties of dried EBN in terms of moisture and thermal diffusivity during IR-UVC assisted drying.
- To determine the physiochemical properties such as colour, moisture reabsorption ability and shrinkage of dried EBN in IR-UVC assisted drying.
- To determine the retention of biochemical properties such as sialic acid and antioxidants of dried EBN in IR-UVC assisted drying.
- To reduce the nitrite content of dried EBN from 200 ppm to 30 ppm or below based on Malaysia Standard MS 2509:2012(P) by IR-UVC assisted drying.
- To investigate the storage stability of IR-UVC assisted dried EBN.
- To optimize the intermittent IR-UVC assisted drying of dried EBN by response surface methodology (RSM).

1.4 Research Scopes

- ***Determination of drying characteristics***

EBN were dried by IR assisted drying at low temperature (IR25) (25°C, 26.7% RH and intermittency, α = 1.00), UVC assisted drying at low temperature (UVC25) (25°C, 26.7% RH and α = 1.00) and IR-UVC assisted drying (IR-UVC25 and IR-UVC40) (25°C-40°C, 16.5%-26.7% RH and α = 0.20-1.00) and hot air drying (HA80) (80°C, 8.3% RH and α = 1.00), respectively. Drying kinetics and drying rates were examined based on the moisture content profile. Fan assisted drying (FAN27) (27°C, 39.7% RH and α = 1.00) was used as reference method.

- ***Determination of mass transport and thermo-physical properties***

Equilibrium moisture content and water activity were measured at the end of drying. The effective moisture diffusivity, D_{eff} was determined from sample moisture based on Fick's second law of diffusion. The effective thermal diffusivity, α_{eff} was estimated by taking sum of the fractional contributions made by the proximate composition of each pure component (moisture, protein, carbohydrate, ash and fibre) in dried EBN samples. Comparison was made against values obtained from hot air drying (80°C).

- ***Evaluation of colour alteration***

Colour analysis was performed to quantify the extent of colour changes in IR assisted drying, UVC assisted drying and IR-UVC assisted drying, respectively. Comparison was made against hot air dried samples and commercial EBN samples (by fan assisted drying).

- ***Evaluation of moisture reabsorption ability and shrinkage***

Dry basis holding capacity (DHC) index, water absorption capacity (WAC) index, moisture reabsorption ability (RA) and shrinkage analysis were performed after IR assisted drying, UVC assisted drying and IR-UVC assisted drying, respectively. Comparison was made against hot air dried and commercial samples (by fan assisted drying).

- ***Evaluation of nitrite content***

Nitrite test method was carried out based on Malaysia Standard MS 2509:2012(P) (Department of Standards Malaysia, 2012). High capacity anionic exchange column compatible to gradient elution, such as Dionex IonPac As 14a with a conductivity detector was used in the analysis of anion content. Comparison was made against hot air dried and commercial EBN samples (by fan assisted drying).

- ***Evaluation of sialic acid and antioxidant retention***

EBN samples were subjected to sialic acid analysis by HPLC and total antioxidant activity (TAC) analysis by using three in-vitro chemical methods, which are 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay, 1,1-diphenyl-2-pycrylhydrazyl (DPPH) free radical scavenging assay, and Ferric reducing antioxidant power (FRAP) assay. Comparison was made against hot air dried and commercial EBN samples (by fan assisted drying).

- ***Investigation of dried product storage stability***

Nitrite content of EBN stored in the UV assisted storage cabinet with storage environment controlled at 27°C and 28.9% RH (method 1) was fabricated to store EBN. Stability of final product was monitored by following the water activity profile and colour degradation kinetics throughout the six-month storage period. Comparison was made against another two current storage methods that are being practised by operators in the industry such as by storing in a closed plastic container and refrigerate at -2°C (method 2) and vacuum sealed plastic packed by vacuum packing machine (model TVPM-400, Euroasia Food Equipment Sdn. Bhd., Penang, Malaysia) (method 3). During storage, colour analysis was performed in monthly basis. At the end of storage, samples were analysed for final moisture content, water activity, colour changes, nitrite content, yeast and mould contents, sialic acid and antioxidant contents.

- ***Optimization of IR-UVC assisted intermittent drying***

The purpose of optimization by response surface methodology (RSM) was to design an intermittent drying profile that gives the shortest drying time, maximum retention of sialic acid and antioxidant and better product colour with nitrite content less than 30 ppm. The drying variables were intermittent duration (X_1), intermittent ratio (X_2) and intermittent cycle (X_3); while the response variables were total drying time (Y_1), total heating time during intermittent (Y_2),

total colour change (Y_3), nitrite content change (Y_4), sialic acid content (Y_5) and antioxidant content (Y_6). A three level experimental design proposed by Box and Behnken (1960) was applied for response surface fitting, with a design matrix of seventeen trials.

1.5 Significance of Study

In this research, the current issue of bleaching EBN could be eliminated. Besides, EBN can be processed at the shortest processing period to significantly reduce the nitrite content less than 30ppm by avoiding the multiplication of nitro-bacteria which generates nitrite compounds while maintaining the cleaned dehydrated EBN safe and hygienic. This in turn ensures high quality and safety of the processed EBN. Furthermore, study on the stability of the dried product during storage period is important in providing valuable information on the changes of colour, sialic acid, antioxidant and nitrite contents during postharvest handling of dried EBN. In-depth knowledge in the drying and quality degradation characteristics of EBN enables design of economically feasible dryer for the industry. Dried EBN with high content of natural antioxidants and sialic acid as well as low nitrite content will add value to the nutraceutical properties of the EBN and enhance the commercial value in the global market.

The findings from this research will greatly benefit the industry and many EBN farmers, traders and exporters that are affected by the recent nitrite contamination. By solving these major issues facing by the industry, the price of EBN may be strengthened and this in turn contributes significantly to the Gross National Income (GNI). In overall, the current study contributes to the knowledge enrichment on the processing aspects of EBN. A better and advanced drying method (IR-UVC assisted drying) is therefore recommended to the EBN industry and serves as a gateway towards the use of advanced drying technology to produce high quality cleaned and dried EBN.

CHAPTER 2 LITERATURE REVIEW

2.1 Swiftlet Farming and Edible Bird's Nest

Edible bird's nest (EBN) (known as "Yan Wo" in Chinese, "Sarang Walet" in Indonesian and "Enso" in Japanese) refers to the nest produced by the swiftlet. More than 24 species of insectivorous and echolocating swiftlets live around the world, but only a few swiftlet species produce nests that are deemed 'edible', which include the White-nest swiftlet (*Aerodramus fuciphogus)* and the Black-nest swiftlet (*Aerodramus maximus)* (Koon, 2000). Majority of EBN traded worldwide come from these two heavily exploited species. They are mainly restricted to the tropical and sub-tropical regions extending from the western Indian Ocean (i.e. Seychelles Islands) through southern continental Asia, Indonesia, Palawan in the Philippines, northern Australia, New Guinea and the islands in south-west of Pacific (Chantler and Driessens, 1995).

The main producers of EBN in commercial quantities include Indonesia (i.e. Sumatra, Java, Kalimantan and Lesser Sunda Islands), Thailand, Malaysia (i.e. Kelantan, Terengganu, Selangor, Perak, Sabah and Sarawak), Vietnam and Myanmar (Koon, 2000; Koon and Cranbrook, 2002). Although there are swiftlets colonies in Hainan Island in China, Andaman and Nicobar Island in the Indian Ocean (Sankaran, 1995; 1998), the production is small and insignificant compared to the Association of Southeast Asia Nation (ASEAN) countries. Therefore, from this context alone, Malaysia is positioned in a strategic geological region in term of swiftlets distribution.

Normally, male swiftlet builds nests (Sankaran, 2001). During nesting and breeding season, reproduction of nests will increase due to the swiftlet's sublingual salivary glands that increase their weight from 2.5 mg to 160 mg and

reach their maximum secretory activity (Medway, 1962). The white nest is made almost entirely from saliva (Sims, 1961) while the black nest contains about 10% feathers with 8% of the protein content in the nest and then the swiftlets will attach it to the vertical walls of inland or seaside caves (Kang et al., 1991). In order to support the mother and the nestlings, the weight of the nest must be 1-2 times the body weight of the swiftlets. The swiftlets normally take about 35 days to construct a nest (Marcone, 2005).

Human consumption of these nests has been a symbol of wealth, power and prestige. It is being used medicinally in traditional Chinese medicine and Chinese cuisine dating as far back as the Tang (618 – 907 AD) and Sung (960 – 1279 AD) dynasties (Koon and Cranbrook, 2002). Since ancient times, Chinese have cooked the tonic food which is well known as "bird's nest soup" as shown in Figure 2.1. It is generally believed by the Chinese community that EBN has a high medicinal value, such as immuno-enhancing, for supplementing human health.

Figure 2.1 Traditional Chinese medicine and cuisine – "Edible Bird's Nest Soup".

Undoubtedly, nest harvesting is normally carried out by local people according to a complex system of ownership and management (Hobbs, 2004; Koon and

Cranbrook, 2002). The unique technique of harvesting EBN is required by the collectors and is influenced by the cave site and height above the ground or water bed (Leh, 2001). The collectors might need to use temporary scaffoldings made of locally collected bamboo or iron-wood if the bird's nest is built high on the cave walls (Marcone, 2005). Sometimes, the cave walls are very slippery so nest harvesting is a very painstaking and dangerous process.

Due to high nutritional values of the nests and the dangers that might be faced during collection, EBN is therefore one of the world's most expensive animal products costing 2,000-10,000 USD per kilogram. Also, its market price has been growing up significantly in the past (Hobbs, 2004; Koon and Cranbrook, 2002; Sankaran, 2001). Besides, illegal developers have been cutting down forests for housing and industrial developments that is affecting the ecosystem and causing a decline in swiftlets feed. Koon and Cranbrook (2002) argued that swiftlet species and its nests might die out in five to ten years if continuous harvesting is being carried out at its current rate. As swiftlet populations is under threatening and this has led the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) to consider adding swiftlets and its nests into its lists of endangered species (Koon and Cranbrook, 2002).

Today, harvesting pressure has increased throughout the years, with the eggs and chicks are being destroyed at the time of collection. Consequently, some populations of swiftlets have marked huge declines and extinction (Hobbs, 2004). Lau and Melville (1994) reported that a decline of about 48% in the population of black-nest swiftlets at Niah between 1935 and 1987, and in Baram, a decline of about 43% in the population of EBN over 17 years (Lau and Melville, 1994). With an attempt to recover the swiftlets populations in April 1989, the Sarawak government announced a three-year ban on nest-collection at Niah Cave that contains the largest swiftlet colony in the world.

To transform the dangerous nest harvesting task, nest harvesters in Indonesia have used houses and develop the practice of swiftlets farming. This has been practiced in recent years by many nest harvesters in Indonesia (60%), Thailand (20%), Malaysia (10%) and Vietnam (7%) (Kuan and Lee, 2005). In swiftlets farming, the farmers build structures to create a cave like atmosphere which is conducive to the swiftlets and so called bird's house. The structures of bird's house come in various shapes and sizes, made from bricks, cinder blocks, cement and wood, even roof sheeting and plywood to keep the capital costs low. As more people get involved, bird's houses are converted from shop houses nowadays, where windows are sealed and ceilings are modified to create nesting space. Others also build simple structures at the residential or vacant land. These privately owned buildings can cost from a few thousand up to hundreds of thousands of ringgit Malaysia (ICOTOS, 2011).

Swiftlet farming industry in Malaysia has been growing fast over the last eight years. Before 1998, there was only an estimated of about 900 swiftlet farms throughout the country. By 2006, the number of swiftlet farms throughout the country was about 36,000 units, with an average annualized growth rate of 35% per year in the last five years (Merican, 2007). The quality of raw bird's nests is very important to the swiftlet farming industry as it has a direct impact on the income of swiftlet farmers. Rearing and managed husbandry of swiftlets in a well-equipped and highly productive avian farm or EBN production facility dedicated to producing EBN on a commercial scale will prevent extinction and enhance the survival of wild avian species by providing an alternative supply of farmed EBN in the market, and reducing as well as stabilizing prices of EBN (Yik, 2011).

2.1.1 Types of edible bird's nest

Edible bird's nests (EBN) are graded according to the location where the nests are built (Table 2.1), edibility, external features, colour, quality (Table 2.2) and

cleanliness. It can be sorted into two main categories: cave EBN and farm EBN as shown in Figures 2.2 and 2.3, respectively. The end products of EBN can be whole piece, narrow piece and biscuits as shown in Figure 2.4.

Figure 2.2 Cave edible bird's nest

Figure 2.3 Farm edible bird's nest

Cave EBN refer to the nests built by swiftlets in natural stone walls or limestone caves. These bird's nests absorb many minerals from the stones or caves. The colour of the nests is usually beige or yellow and they are more solid compared to farm EBN. They can be stewed for longer hours as compared to farm EBN. These nests are delicious and taste better after stewing. They are able only to expand 2-3 times upon soaking. However, cave EBN contain more fine feathers and impurities compared to farm EBN (Jong et al., 2013).

Meanwhile, farm bird's nest are built in swiftlets houses. Swiftlets build their nests on the beams and walls in the houses, thus resulting in a slight taste of sawdust in the bird's nests. The workers have to check the bird's nests on a regular basis and will harvest the bird's nests every three months. After collection, the bird's nests will be cleaned to remove feathers and impurities. Farm EBN are higher in quality as they have less feathers and less impurities. As compared to cave EBN, farm EBN expands better in water and on the average, farm EBN expand 5-7 times of original size. Farm EBN taste smoother in texture but cannot be stewed for long hours (about 30-45 minutes before ready to be served) (Jong et al., 2013).

Table 2.1 Quality, production volume and market price of edible bird's nest in different geographical conditions (Jordan, 2004; Kuan and Lee, 2005; Marcone, 2005; Merican, 2007).

Country	Quality	Production Volume	Market Price
Indonesia	Smoother and softer in texture; cleaner with fewer impurities	Largest producer approximately 80% of the world supply	Moderate (<RM 3,000/kg)
Thailand	Thicker and firmer with higher density	Small production	Moderate (<RM 3,000/kg)
Malaysia	Smooth and soft in texture; more impurities	Large production	Lowest (<RM 2,000/kg)
Vietnam	Smoother and softer in texture; cleaner with fewer impurities; richer taste and aroma	Very small production	Highest (RM 3,000-4,000/kg)

Table 2.2 Qualities of farm and cave edible bird's nests (Koon and Cranbrook, 2002).

Swiftlet Types	White nest swiftlet (*Aerodramus fuciphagus*)	Black-nest swiftlet (*Aerodramus maximus*)	Grass swiftlet (*Collocalia esculenta)*
Nest Types	House or cave nest	Cave nest	House or cave nest
Percentage of Edible	90%	50%	10%
Processing	Remove minor amount of feathers by forceps and molded into nest or cake-shapes	Remove many feathers and impurities by machines and molded into nest or cake-shapes	Remove grass, feathers and impurities by forceps and molded into nest or cake-shapes
Colour	White and golden	White and yellow	Green and yellow
Texture	Smooth and soft after stewing	Crispier texture after stewing	Smooth and soft after stewing; contain little fiber of fresh grass when chewing
Nutritional value	High	Moderate	Low

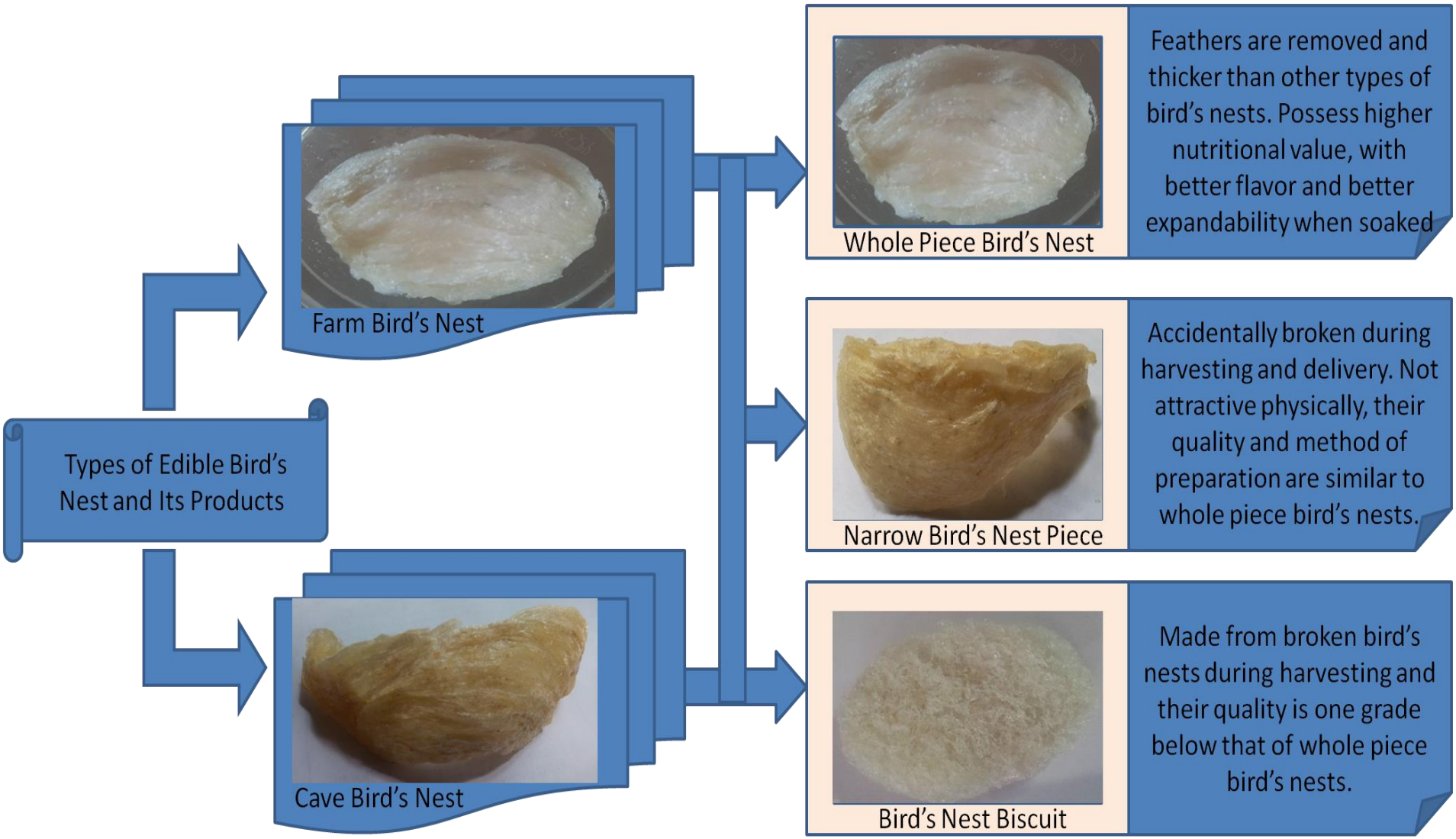


Figure 2.4 Types of edible bird's nest final products.

2.1.2 Grading of edible bird's nest

Apart from the appearance, the EBN are graded according to the harvesting period, shape, cleanliness, size, colour, density and expansion coefficient. Preservation of EBN to its original shape command the highest price followed by the size and cleanliness of EBN (Tan et al., 2014). In terms of harvesting period, especially at the first stage of bird's nests, due to abundant rainfall from January to April each year in Malaysia, this is an excellent time for propagation of small animals and plants. With sufficient food and good health conditions, swiftlets are able to produce more saliva during this period. Thus, bird's nests contain less foreign matters and the bird's nest pieces produced are larger and thicker (Ma and Liu, 2012).

Second and third stages of bird's nests harvested from May to December each year in Malaysia, where there is lesser rainfall due to changes in seasons and this results in a reduction in food sources for the swiftlets. During dry season, raw bird's nests harvested are found to be dirtier and contain more impurities such as feathers and other elements as compared to those harvested in wet season. That is why the bird's nests are loose (less firm) and the bird's nest pieces are less attractive. Thus, the price of first stage bird's nests is the highest as compared to the second and third stages of bird's nests (Ma and Liu, 2012).

In terms of shape and size, the most preferred shape is a perfect half cup that is large but not too thick and without holes. Most of the raw bird's nests are subdivided into three categories, which are grade 'A', grade 'B' and grade 'C' as shown in Figure 2.6. The shape of grade 'A' nests resembles a half-cup shape with 180° or even surface when placed on the horizontal surface. Grade 'B' nests have similar shape as those of grade 'A' but it is 135° when placed on flat surface. Grade 'C' is only half of grade 'A' and it is 90° when placed on a flat surface as shown in Figure 2.6. Grade 'A' nest would have a minimum measurement of W =

3.5 cm x L = 7 cm and grade 'B' would have a measurement of W = 2.5 cm x L = 5 cm. Meanwhile, grade 'C' would have a measurement of W = 1.5-2 cm x L = 3.5-4.5 cm. In most of the new swiftlet farm, the swiftlet would occupy the four corners of the wooden batten as nestling place. This is due to location in which it is easier for the birds to construct nest with lesser resources (Tan et al., 2014).

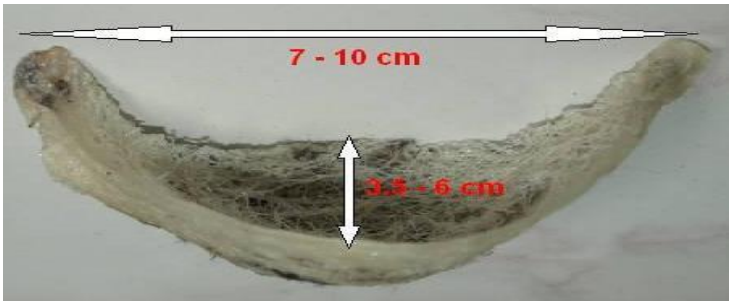


Figure 2.5 Measurement method of an edible bird's nest.

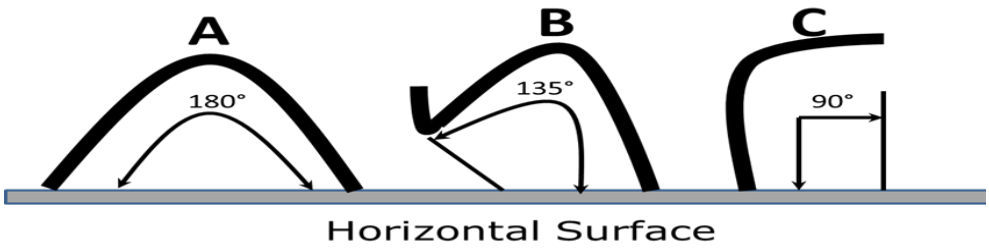


Figure 2.6 Grading of raw bird's nests.

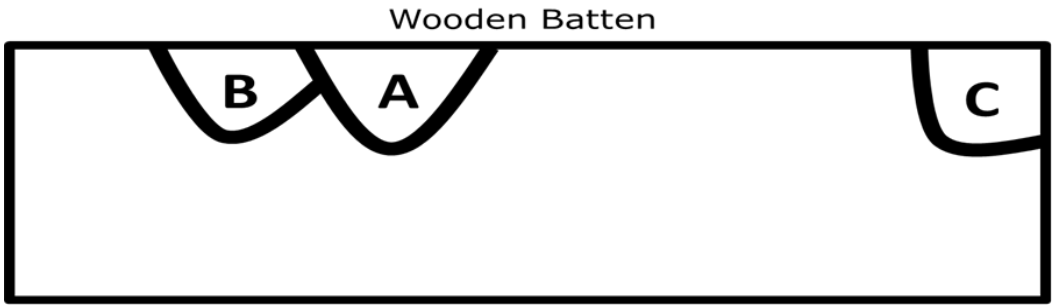


Figure 2.7 Formation of raw bird's nests on ceiling section in swiftlet farms (Tan et al., 2014).

Moisture content in the bird's nest also greatly affects its shape i.e. when too dry, the bird's nest tends to be very brittle. Spraying more water could preserve the shape and prevent the bird's nest from breakage during transportation. However, the usual practice only allows for 10% of moisture contents. The main export market specifies moisture content of 10% in Hong Kong but some others allowed for higher moisture content such as in Taiwan. This has become controversial as some owners tried to increase the total weight by spraying more water on the nests (Tan et al., 2014).

Cleanliness is also one of the determining factors on the value of EBN. The farmers have to remove all traces of feathers and impurities to obtain higher market price. Regular cleaning of guano from swiftlet farms could reduce the impurities. However, the moult seasons of swiftlet from May to December greatly affects the cleanliness of EBN due to the presence of more shredded feathers in the raw-unclean EBN (Tan et al., 2014).

Lastly, white colour EBN has been recognized as the highest grade with highest market price, followed by mixture of more yellowish nests. The yellowish colour may be due to high moisture inside the bird's house and high concentration of guano which contains nitrite compound (Ma and Liu, 2012; Tan et al., 2014). Different types of mineral are contained in the food which could cause the saliva secreted by swiftlets to give different shades (Ma and Liu, 2012).

Despite the rapid growth of EBN demand and value, scientific investigations on its medicinal and nutritional properties are still very limited, especially given the fact that these properties appear to vary with the time of harvest and location. This has opened up opportunity for adulteration during processing with less expensive materials such as karaya gum, red seaweed, fried pork skin, egg white, gelatin, soybean, rice, starch, agar, fish vesicae and *Tremella* fungus. Also, other

methods such as staining, bleaching and incorporating cheaper EBN into the more expensive ones have been reported (Food Quality News, 2011; GuangZhou News, 2011; Xinhuanet, 2012; ZheJiang News, 2011). The difference between chemically treated processed and untreated EBN can be easily discovered by the glossiness of the cement strands after soaking in water for 30 minutes.

Table 2.3 Accurate techniques to differentiate between pure and fake edible bird's nests (Wu et al., 2007; Yang et al., 2013).

	Characteristics	Real Edible Bird's Nest	Fake Edible Bird's Nest
Before soaking in water	Transparency	Semi-transparent	Not transparent and might be reflective
	Fragility	Very fragile and breaks into small pieces	Very fragile but breaks into larger chunks and never in small pieces
After soaking in water for 30 minutes	Shape and Appearance	No distinctive shape, easily recognizable with an occasional feather stuck within the nest fibers	Bumpy surface arranged uniformly with slight smell of medication
	Expansion coefficient	Expand to 2-3 times (cave EBN) or 5-7 times (farm EBN) its original size	No significant expansion. Evident sign of foreign particles
	Colour	Soaking water remains clear	Discolouring of nest colour into water due to artificial colourings
	Presence of foaming	Stirring of bird's nest in water causes foaming to appear on the surface, while water remains clear throughout	Stirring does not produces foaming, water remains murky and clouded
	Structural Observation under Scanning Electron Microscopy (SEM)	Unevenly structured	Coarsely distributed
	Environmental Scanning Electron Microscopy (ESEM)	Presence of five monoses and epidermal growth factor (EGF)	Absence of five monoses and epidermal growth factor (EGF)

2.1.3 Composition of edible bird's nest

EBN are composed mainly of glycoproteins; in turn, EBN has the properties of protein as well as of carbohydrate. Many studies have been conducted to define the precise roles of oligosaccharides chains in the functionality of glycoprotein. The carbohydrate part of the glycoprotein contains galactose, mannose, glucosamine, galactosamine and sialic acid. Several glycoprotein functions have been identified but many are still under investigation. As EBN consists of main types of glycoprotein, it can be used as lubricant and protective agents (Marcone, 2005).

The carbohydrate component in fresh EBN consists of 9% sialic acid, 7.2% galactosamine, 5.3% glucosamine, 10.9% galactose and 0.7% fucose (Kathan and Weeks, 1969). As compared to pure proteins, EBN has a higher percentage of humin nitrogen and cysteine nitrogen which may be due to the carbohydrate radical and fine feathers present in the bird's nest. The most abundant amino acids present are serine, threonine, aspartic acid, glutamic acid, proline and valine (Chen et al., 1996; Wang, 1921a). Two aromatic amino acid rich in the white bird's nest are phenylalanine and tyrosine, which are not usually present in most plant proteins (Ma and Liu, 2012).

Moreover, the two main functional components in EBN found by many researchers are sialic acid and epidermal growth factor (EGF). It was also found that sodium, magnesium, potassium and calcium are the major minerals content in EBN. These minerals are required to activate thousands of enzyme reactions within the body. For example, sodium balances the electrolyte, body fluid volume, maintain the nerve impulse condition; while, magnesium maintains the alkaline balance and control of neuromuscular activity (Marcone, 2005). In a recent research by Ma and Liu (2012), six types of hormones that exhibit important

bioactivities in EBN were extracted and have been determined to be very important in human health.

2.1.4 Health Benefits of Edible Bird's Nest

In general, EBN is recognized and believed by the Chinese community that it is a highly valuable food tonic due to its high medicinal value. A number of claimed benefits of consuming EBN includes dissolving phlegm, relieving gastric discomforts, aiding renal functions, raising libido, enhancing complexion, alleviating asthma, suppressing cough, curing tuberculosis, strengthening the immune system, speeding recovery illness and surgery, and increasing energy and metabolism (Konga et al., 1987; Ma and Liu, 2012). EBN is particularly suitable for pregnant women, infants, the aged, or anyone suffering from weakness or poor digestion (Yang et al., 2013).

It is suggested that EBN might possess immuno-enhancing effect by aiding cell division of immune cells (Biddle and Belyavin, 1968; Zhang et al., 1994). Lymphatic cells are important to immune cells in the human body and can divide into T lymphatic cells and B lymphatic cells. The mitochysis substance contained in bird's nest stimulates lymphatic cells to improve immune system in the human body. EBN extract could neutralize infection with influenza viruses in Madin-Darby canince kidney (MDCK) cells and inhibit the hemagglutination of influenza A viruses (such as human, avian, and porcine strains) to human erythrosytes. It is also one of the basic substances contained in edible bird's nest for supplementing human health (Biddle and Belyavin, 1968; Zhang et al., 1994).

Moreover, Kyung et al. (2012) demonstrated an epidermal growth factor (EGF)-like activity in aquoues extract of EBN that stimulated the DNA synthesis in 3T3 fibroblast in a dosedependent manner in vitro. EGF is a 6,000 Da polypeptide

hormone produced by glands of the gastrointestinal tract, namely the salivary and Brunner's glands. It plays a crucial role in the cellular processes such as proliferation, differentiation and development in ageing resistance. Chua et al. (2013) also claimed that EBN extract can reduce the progression of osteoarthritis, help in the regeneration of the cartilage and improve the bone strength (Matsukawa et al., 2011). Matsukawa et al. (2011) concluded that EBN extract could improve the bone strength and dermal thickness in ovariectomized rats without increasing the level of serum estradiol (E_2) which may increase the breast cancer risk. This proves that EBN could be used for the improvement of bone strength and skin aging during post-menopause in women.

2.1.5 Processing line of edible bird's nest

As the swiftlet farming industry continues to expand and grow, more and more supplies of EBN sourced from swiftlet farms began to find their ways into the supply chain (from farming to processing line as shown in Figure 2.8). After collection of bird's nest, the raw-unclean EBN shall undergo soaking, wetting, dampening with potable water, and cleaning under the filtered water by a brush and a pair of forceps to wash out some impurities on the surface of EBN.

Next, the strands or broken filaments of EBN are arranged into a mould before drying such as by conventional air drying (by using fan or light bulb), oven drying and freeze drying. Drying should be done in a confined dust free area and at recommended temperature of less than 60°C. Upon drying, grading is carried out before packaging. Packaging should be done in hygiene conditions, controlled temperature and according to grade respectively.

1. Receiving and Sorting

- The raw-unclean EBN are received from the cave or farm.
- The raw-unclean EBN will undergo sorting and grading according to the shape, size, colour and impurities content.

2. Cleaning

- The raw-unclean EBN shall undergo soaking, wetting, dampening with potable water.
- After that, the raw-EBN shall be cleaned with tap water and a brush to wash out some impurities on the surface of EBN.
- Followed by removing the big and small feathers and impurities in the EBN with a pair of forceps.

3. Moulding

- Moulding process shall be carried out in a separate working area that is environmentally controlled, well light and ventilated.
- The strands or broken filaments of EBN are arranged into a mould before sent to drying process.

4. Drying

- The drying methods such as conventional air drying (by using fan or light bulb), oven drying and freeze drying are available in the industry now.
- Drying activities should be done in confined dust free area and at an advisable temperature <60°C.

5. Packaging and Grading

- Grading may be carried out before packaging. Packaging should be done in hygiene conditions, controlled temperature and according to grade respectively.

Figure 2.8 Processing line of edible bird's nest in the industry (from sorting to packaging).

2.2 Drying Methods

Choosing the proper dryer and drying condition are important to ensure good quality of the dried products. There are some negative effects of drying such as loss of nutrients, loss of colour and deformation of internal structure that can be minimized by optimizing the process parameters.

2.2.1 Fan assisted drying

Fan assisted drying is preferred by the small scale EBN industry due to the small drying quantity, low cost and simple equipment setup. Fan provides air circulation to remove surface vapour of the product and allow diffusion of internal moisture to the material surface for further evaporation. Generally, fan assisted drying is operated under ambient temperature (25-27°C), moderate relative humidity (45-55% RH) and high air velocity (4.5-6.0 m/s). Fan assisted drying provides numerous advantages, such as low operating cost, simplicity in operation, great energy savings and space savings as compared to other advanced drying methods. However, the processing time can be extremely long (Banyan Bird Nest, 2010; Borneo Bird's Nest, 2015).

Undeniably, final products obtained from fan assisted drying are often associated with quality defects in terms of poor colour development, inferior texture and noticeable shrinkage. Fan assisted drying is normally carried out in an open environment under room temperature and mould growth could not be prevented when compared with hot air drying. Moreover, relatively long processing time often results in nutrients loss and severe browning in final product (Banyan Bird Nest, 2010; Borneo Bird's Nest, 2015; Dynasty Nest, 2012).

2.2.2 Hot air drying

Hot air drying is the most common drying method used in the agricultural and food industry. Conventional dryers apply primarily hot air where ambient air is

first heated to a desired temperature and then brought into contact with the moist material surface to facilitate moisture removal. Generally, the operating temperature in hot air dryer may range from 40°C-90°C (Doymaz, 2005; Karathanos and Belessiotis, 1999); while, the operating relative humidity may range from 5% RH to 25% RH and air velocity can be regulated from 0.03 m/s to 4.50 m/s (Doymaz, 2005). Hot air drying helps to reduce the drying time and hence limits mould growth when compared to solar drying and provides better hygienic processing environment (Naphaporn et al., 2015). Hot air drying temperature is also limited by the heat sensitivity of the material and expected quality of the final product (Lewicki, 2004).

Numerous research works have been carried out to study drying kinetics and product quality of foodstuffs by conventional hot air drying. Market and industrial demand creates a need to design dryers with high drying rate and produce good quality of dried products. Nonetheless, final products obtained from conventional hot air drying are often associated with poor colour development, inferior texture and noticeable shrinkage. Moreover, relatively high temperature in hot air drying often results in the loss of valuable but heat-sensitive bioactive ingredients and nutrients (Hiranvarachat et al., 2011; Leeratanarak et al., 2006). Summary of dryer settings and product quality of some selected works on conventional hot air drying is as shown in Table 2.4.

Table 2.4 Selected works on hot air drying and product quality.

Product	Dryer settings	Product quality
Crushed Feed Rice (Fumina et al., 2016)	▪ 40°C-70°C ▪ 10% RH	▪ Lysine (the first limiting amino acid in pig nutrition) content in crushed rice decreased with increasing drying temperature
Squid Fillets (Deng et al., 2015)	▪ 60±2°C ▪ 1.50 m/s ▪ 20% RH	▪ Retained least amount of amino acid ▪ Retained lowest amount protein quality and digestibility ▪ Caused severest damage of myosin structure
Algae (Cristian et al., 2011)	▪ 40°C-70°C ▪ 2.0±0.2 m/s	▪ Colour, rehydration indices and texture of dried algae decreased with process temperature ▪ Antioxidant activity and phycobiliproteins were notably influenced by increasing drying temperature
Kiwifruit (Takahiro et al., 2013)	▪ 50-70°C ▪ 0.3 m/s	▪ Severe colour and texture deterioration ▪ Great loss of L-ascorbic acid and antioxidant activity
Chinese Ginger (An et al., 2015)	▪ 60°C	▪ Low retention of chemical profiles, antioxidant activity, cellular structures
Potato (Setiady et al., 2009)	▪ 60°C ▪ 1.8 m/s	▪ Slower rehydration time ▪ Lower water holding capacity (almost twice) compared to freeze dried samples ▪ Severe colour deterioration due to browning compared to freeze dried and microwave vacuum dried samples
Okra (Inyang and Ike, 1998)	▪ 40-80°C	▪ Great colour change ▪ Great loss in ascorbic acid content ▪ Negative effect on viscosity

2.2.3 IR and UVC assisted drying

Infrared radiation (IR) is an electromagnetic spectrum that is predominantly responsible for the heating effect from the sun and has three categories based on its wavelength namely the near-infrared (NIR= 0.78-1.4 μm), middle-infrared (MIR= 1.4-3 μm) and far-infrared (NIR= 3-1,000 μm) (Mongpraneet et al., 2004; Ranjan et al., 2002). The transition of infrared radiation through water is at NIR, whereas at FIR (longer wavelength) it is absorbed at the surface. In turn, the drying of thicker bodies seems to be more efficient using NIR, whereas the drying of thin layers yields better results using FIR (Nuthong et al., 2011). The IR absorption wavelength for various chemical groups and relevant food components are summarized in Table 2.5.

Table 2.5 The IR absorption wavelength for chemical groups and relevant food components (Krishnamurthy et al., 2008).

Chemical group	Absorption wavelength (μm)	Relevant food component
Hydroxyl group (O-H)	2.7 to 3.3	Water, Sugars
Aliphatic carbon-hydrogen bond	3.25 to 3.7	Lipids, Sugars, Proteins
Carbonyl group (C=O) (amide)	5.92	Proteins
Nitrogen-hydrogen group (-NH-)	2.83 to 3.33	Proteins

In recent years, infrared (IR) drying has been applied widely in the food industries because of its numerous advantages such as energy savings, lower drying time, high quality dried products, intermittent energy source, easy control of the process parameters, uniform temperature distribution, clean operational environment and space savings (Sakai and Hanzawa, 1994). The transfer of IR energy is done without heating the surrounding air and no heating medium is

therefore needed between the source of the energy and the material in IR dryers. IR radiation penetrates directly into the inner layer of the material without heating the surrounding air, hence, the energy consumption of infrared drying is lower compared to other drying techniques (Riadh et al., 2015).

A number of drying trials using IR dryer have been conducted in recent years as shown in Table 2.6. IR drying is known as a drying technique that allows high rate of water evaporation without quality losses such as colour changes, shrinkage, surface hardening, sample deformation, loss of aroma and ascorbic acid degradation (Riadh et al., 2015). IR heating can also be used to inactivate bacteria, spores, yeast and mould in both liquid and solid foods. The efficacy of microbial inactivation by IR heating depends on the following parameters namely IR power level, temperature of food sample, peak wavelength, bandwidth of IR heating source, sample depth, types of microorganisms, moisture content and types of food materials (Riadh et al., 2015). Therefore, several researchers have investigated the effects of these parameters on the inactivation of pathogenic microorganisms as shown in Table 2.7.

In terms of heat transfer, vapour flux is one of the main input parameters that affect the performance of the infrared drying system and the effective moisture diffusion coefficient increases with temperature. Ragab et al. (2011) claimed that moisture diffusion coefficients during the heating and cooling of infrared with tempering were higher than those in convective heating. Although IR drying is known to be a promising method, it has limitations in its penetrating power and not applicable for all drying systems. As a result, combination of electromagnetic radiation and other drying methods such as hot air, microwave, freeze drying and UVC irradiation, depending on the specific process, is usually applied to ensure better drying efficiency and better product quality (Riadh et al., 2015). Recently, ultraviolet (UV) irradiation has also been used in indoor environment to eliminate

or control infectious diseases in food production. UV irradiation (wavelength: 100-400 nm) is a type of electromagnetic irradiation, which is divided into four distinct spectral areas including UVA (315-400 nm), UVB (280-315 nm), UVC (200-280 nm) and vacuum UV (100-200 nm) (Dial et al., 2012).

Among these wavelength ranges, UVC has the best potential to inactivate microorganisms because of the strong wavelength (250-270 nm) that is mainly absorbed by the nucleic acids of microbial cells and therefore it is the most lethal range of wavelengths. The bactericidal mechanism of UVC is to cause damage to their RNA and DNA, which often leads to the formation of dimers between pyrimidine residues in the nucleic acid strands. Consequence of this modification could cause defects in cell replication and lead to cell death (Gurzadyan et al., 1995). Studies of the effect of UVC irradiation for inactivation of bacteria or fungi using an UVC dryer is as shown in Table 2.8. Although research has proved that infrared drying can improve quality of heat sensitive high value foods like herbs and UVC can kill bacteria, but there is no report on comparing the drying kinetics and product quality of EBN under low temperature drying with IR and UVC treatment in published literatures.

Table 2.6 Selected works on infrared drying and product quality.

Product	Dryer configurations and settings	Product quality
Squid Fillet (Deng et al., 2014)	▪ Far-infrared assisted heat pump drying ▪ IR power (100, 500 and 800 W) ▪ Air velocity (2.0 m/s) ▪ Air temperature (40°C)	▪ High retention of amino acid ▪ High protein quality of dried samples ▪ Dense and firm microstructure
Peanut (Rim et al., 2005)	▪ Combinations of hot air and far-infrared ▪ FIR wavelength (2 – 14 μm) ▪ Air temperature (150°C)	▪ High retention of total phenol contents ▪ High retention of radical scavenging activity (RSA) and reducing power ▪ Increased water hydration rate
Fresh longan fruit (Nuthong et al., 2011)	▪ Combinations of hot air and IR drying ▪ IR power (300, 500 and 700 W) ▪ Air velocity (0.5, 1.0 and 1.5 m/s) ▪ Air temperature (40, 60 and 80°C)	▪ Increased drying rate ▪ Reduced drying time ▪ Improved effective diffusivity ▪ Improved mass transfer coefficient
Potato Chips (Supmoon and Noomhorm, 2013)	▪ Air jet impingement combined with infrared drying ▪ IR intensity (0.16, 0.27 and 0.33 W/cm^2) ▪ Air velocity (5, 10 and 15 m/s) ▪ Air temperature (85°C)	▪ Higher drying rate ▪ Less shrinkage ▪ Lower hardness ▪ Less colour deterioration
Blueberry (Shi et al., 2008)	▪ Combinations of hot air and IR drying ▪ IR intensity (4,000 W/m^2) ▪ Air velocity (4 m/s) ▪ Air temperature (60, 70, 80 and 90°C)	▪ Higher effective diffusivity ▪ Higher activation energy ▪ Higher drying efficiency ▪ Much firmer-texture product
Barley (Afzal et al., 1999)	▪ Combined far-infrared convection drying ▪ IR intensity (0.167, 0.333 and 0.7 W/cm^2) ▪ Air velocity (0.3, 0.5 and 0.7 m/s) ▪ Air temperature (30, 40, 55 and 70°C)	▪ Faster drying rate ▪ Reduced energy consumption ▪ Obtained high quality of dried products

Table 2.7 Inactivation of pathogenic microorganisms by infrared heating in IR dryer.

Food / Non-food	Surface temperature/ Energy	Time	Pathogen	Log reduction (log_{10} CFU/mL)	References
Wheat	2.0 kW	63 s	Natural microflora	Approx. 2.0 log_{10} CFU/mL	Uchino et al., 2000
Wheat or Soybean Surface	1.5 kW	10 s	Total bacteria count	Approx. 3.0 log_{10} CFU/mL	Daisuke et al., 2001
Turkey Frankfurters	70°C	82.1 s	*Listeria monocytogens*	3.5 ± 0.4 log_{10} CFU/cm^2	Huang, 2004
	75°C	92.1 s	*Listeria monocytogens*	4.3 ± 0.4 log_{10} CFU/cm^2	
	80°C	103.2 s	*Listeria monocytogens*	4.5 ± 0.2 log_{10} CFU/cm^2	
Egg shells	70°C	1.5 s	*Salmonella enteritidis*	Up to 6 log_{10} CFU/mL (estimated)	James et al., 2002
Nutrient agar (depth = 0 mm)	4.36 kW	6 min	*E.coli*	Approx. 2.30 to 2.48 log_{10} CFU/plate	Hashimoto et al., 1992b
Nutrient agar (depth = 1 mm from surface)	4.36 kW	6 min	*E.coli*	Approx. 0.70 log_{10} CFU/plate	
Nutrient agar (depth = 2 mm from surface)	4.36 kW	6 min	*E.coli*	Approx. 0.66 log_{10} CFU/plate	

Table 2.8 Studies of the effect of UVC irradiation for inactivation of bacteria or fungi *in vitro* by an UVC dryer.

Light source	Radiant Exposure	Bacteria / fungi species / strains	Inactivation efficacy	Reference
254 nm UVC	5 mW/cm^2	MRSA, *Streptococcus pyogenes*	Illuminated 5 s, methicillin-resistant, coagulase-negative *Straphylococcus*and *Streptococcus pyogens*inactivation; illuminated 15 s, methicillin-susceptible *S. aureus* and *Enterococc*i species inactivation	Su et al., 2011
265 nm UVC	1.93 mW/cm^2	*S. aureus, E.coli, Pseudomonas aeruginosa, S. pyogenes*	Illuminated 1 s, 100% inhibition for all strains	Petty et al., 2011
254 nm UVC	1500 mW/cm^2	Catheter biofilms of *E. coli,* coagulase-negative *staphylococcus, E. faecalis, Streptococcus, P.aeruginosa, Coryneforms*	Mean killing rates of the bacteria in catheter biofilms were 89.6% (11.8 mJ/cm^2), 98% (47 mJ/cm^2) and 99% (1500 mJ/cm^2)	Ladefoged et al., 2009
254 nm UVC	1500 mW/cm^2	*Trichophytonrubrum, T. mentagrophytes, Epidermophytonfloccosum, Microsporumcanis*	3 – 5 $\log_{10}$ of fungal inactivation	Dai et al., 2008
254 nm UVC	15.54 mW/cm^2	Bacteria (*Nitrobacter, P. aeruginosa* and *Mycobacterium abscessus)* and fungi (*Candida albicans, Aspergillus fumigates)*	Illuminated 3-5 s, 99% bacteria inactivation; illuminated 15-30 s, 99% fungi inactivation	Sullivan and Conner, 2000

2.3 Intermittent Drying Scheme

2.3.1 Intermittent drying

Drying is an energy intensive operation that can consume up to 15% of all industrial energy usage, often due to low thermal efficiency of dryer in the range of 25-50% (Chua et al., 2001) and improving energy efficiency by 1% could result in as much as 10% increase in profit (Beedie, 1995). Drying causes changes in the food properties including loss of nutritive value and aroma, discolouring, textural changes and changes in physical appearance and shape (Quirijins, 2006). Hence, intermittent drying has been recognized as one of the most energy efficient drying processes due to shorter effective drying time, hence lower drying cost, and better product quality (Kowalski and Pawlowski, 2011a). Intermittent drying is a drying method where drying conditions change with time and different drying schemes as explained in Table 2.9.

Table 2.9 Time-dependent drying schemes (Chua et al., 2001).

Category	Definition
Intermittent drying	Heat is supplied intermittently rather than continuously. This can be done by interrupting the air flow to provide the material a "rest" period, by a continuous air flow periodically heated, or by periodic variation of air flow.
Dryaeration	Combination of high temperature short drying period, tempering, and a slow cooling concluded by final drying.
Air Reversal Drying	A drying process that reversing the direction of the airflow for a period of time and then revert it back to its original direction. This is applied to deep bed drying of particulates.
Cyclic Drying	A drying process whereby the air temperature, humidity or even velocity undergoes a specified cyclic pattern variation such as sinusoidal, square-wave or saw-tooth patterns.

Intermittent drying can be accomplished by controlling the supply of thermal energy, which can be achieved by altering the air flow rate, air temperature, humidity or operating pressure. One can also vary the mode of energy input (e.g.

convection, conduction, radiation or microwave) to achieve intermittency as shown in Figure 2.9. These intermittent processes generally showed improvement in quality of dried food when compared with continuous drying.

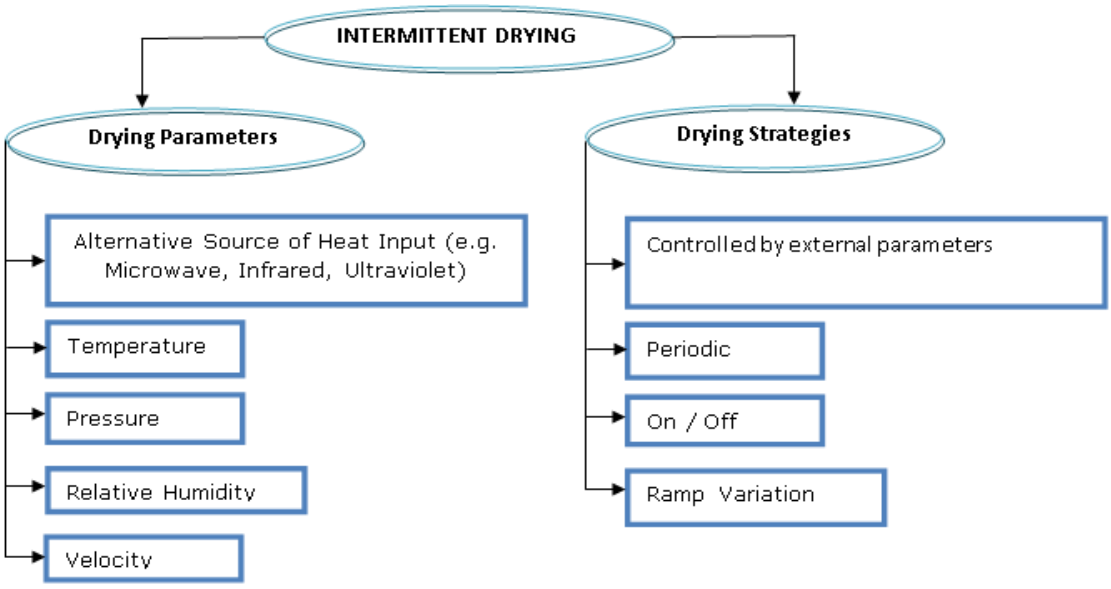


Figure 2.9 General classification schemes for intermittent drying.

During continuous drying, similar amount of energy is supplied throughout the drying process that could result in quality degradation, heat damage to the surface and wastage of heat energy. This is because in later stage of drying, drying rate decreases as sample surfaces do not contain sufficient moisture to be removed. Sample surface becomes dry towards the later stages of drying and constant use of high air temperature causes greater quality degradation. The strategy of using intermittency allows time for moisture to diffuse from the centre to the surface during tempering period. Therefore, quality degradation and heat damage can be minimized by applying intermittent drying.

The most common type of intermittency investigated is the on/off strategy, where heat source is periodically turned on and turned off. Table 2.10 shows a comparison in energy savings by on/off intermittency reported by researchers. Most of the researchers defined the intermittency ratio, α as the ratio of on period of each cycle to total drying time, i.e. $\alpha = \frac{T_{ON}}{T_{ON}+T_{OFF}}$ where T_{ON} and T_{OFF} are the on and off period of each cycle, respectively. For example, α = 0 refers to continuous drying and higher intermittency, α refers to higher tempering period. Jumah (1995) investigated the energy saving by applying different intermittency, α in grain drying in a novel rotating jet spouted bed. The most remarkable finding of this study was that the higher intermittency (higher tempering period), the higher the energy savings. For example, energy savings were 19%, 23% and 30% for tempering periods 10 min (α=0.33), 20 min (α=0.50) and 40 min (α=0.67), respectively. The maximum energy saving of 37% was achieved for 60 min tempering periods (α=0.75). Increasing intermittency, α beyond 0.75 would increase the energy savings but total drying time would be significantly longer (Jumah, 1995).

In facts, longer total drying time may not be suitable to some products as this could result in quality deterioration. Therefore, the intermittency should be selected carefully in order to get optimum energy savings. Chin and Law (2010) studied the effect of intermittency on quality and drying kinetics of *Ganoderma tsugae*. It was observed that, for the same intermittency (e.g., α=0.33), the energy saving increased from 14% to 21% as drying temperature increased from 28.4°C to 40.6°C. Instead of applying intermittency throughout the drying period, only one or two tempering periods have also been investigated. Holowaty et al. (2012) showed that drying of yerba mate branches with one or two tempering periods could save drying time of 15 min and 30 min, respectively, to achieve similar final moisture content.

Table 2.10 Energy savings by intermittent drying for different intermittency ($\propto$).

Product	Drying Period, T_{ON} (min)	Intermittency, $\propto$	Energy Savings over Continuous Drying (%)	Drying Air Temp & Final Moisture Content	Reference
Grain	Continuous	0	-	80°C and	Jumah, 1995
	20	0.33	19	13% db	
	20	0.50	23		
	20	0.67	30		
	20	0.75	37		
Ganoderma tsugae	Continuous	0	-	28.4°C and	Chin and Law, 2010
	60	0.67	40	11.5% db	
	60	0.80	43		
	120	0.33	13		
	Continuous	0	-	40.6°C and	
	60	0.67	52	8% db	
	60	0.80	52		
	120	0.33	21		
Chinese Cabbage	Continuous	0	-	28.4°C and	Yang et al., 2013
	400	0.50	31	11.5% db	
	400	0.67	48.1		
	800	0.33	24.4		
Squash Slice	Continuous	0	-	100°C and 14.7% db	Pan et al., 1998
	40	0.88	36		
Yerba Mate (Twigs & Leaves)	Continuous	0	-	100°C and 16.8% db	Santiago et al., 2012
	15	0.50	10		
Paddy	Continuous	0	-	100-110°C and	Igathinathane et al., 2008
	90	0.7	45.1	12-14% db	

Another advantage of intermittent drying is the moisture levelling or moisture uniformity in the samples. During continuous drying, similar amount of energy supply throughout drying and drying rate decreases in the later stage of drying as material surface contain lesser moisture for further removal. The surface of samples becomes dry and constant use of high temperature results in quality degradation and heat damage to the surface as well as wastage of heat energy (Zeki, 2009).

According to Jumah et al. (2007), experimental investigation of olive cake drying showed that longer tempering period resulted in more moisture levelling, higher initial moisture removal in each active drying period and more effective energy utilization. Intermittent drying is one of the technical solutions to this problem as intermittency gives time for moisture to migrate from the centre to the surface of the sample during tempering. Hence, intermittent drying can minimize quality degradation and heat damage effectively (Chandan and Mohammad, 2014).

The effect of intermittent drying on other physical changes like cracking and rehydration have been investigated by many researchers. Cracking/brittleness is an important product quality issue for EBN drying. Pan et al. (1998) claimed that the rehydration ability of the product gained by intermittent drying was higher when compared to continuous drying. These improvements could be attributed to the temperature and moisture redistribution during tempering period that helps to reduce temperature and moisture gradient and thus reduces internal stresses. It has been reported that intermittent and time-varying drying offer significant advantages in terms of reducing the required energy and enhancing product quality of heat sensitive product (Chua et al., 2001).

2.3.2 Intermittent application of IR and UVC treatment

To date, infrared (IR) drying has gained popularity for processing heat sensitive high value foods that monitor sample moisture as a measure of quality control (Beary, 1988). Ginzburg (1969) has suggested that infrared (IR) radiation, operating under an intermittent mode, could be applied to dry heat sensitive biomaterials such as grains, flour, vegetables, pasta, meat and fish. Therefore, three drying options are available which are by constantly monitoring the product surface temperature, by regulating the intensity of IR and by operating IR in intermittent mode. According to Ginzburg (1969), the effectiveness of drying increased by applying intermittent IR radiation and also by using combined radiant-convective method.

In many cases, intermittent radiation is beneficial in decreasing duration of the drying process as well as colour change and browning index of the products as shown in Table 2.11. Paakkonen et al. (1999) have shown that intermittent IR drying improved the quality of herbs while Dontigny et al. (1992) has demonstrated that intermittent IR drying of graphite slurry significantly increased drying rate. Zbicinski et al. (1992) investigated both convective air drying and IR drying and suggested the use of intermittent IR radiation drying mode coupled with convective air drying for heat sensitive materials. Other researchers such as Dostie et al. (1989) and Carroll and Churchill (1986) have also reported shorter drying time with improved product quality by using intermittent IR heating (Carroll and Churchill, 1986; Dostie et al., 1989). Zhu and Pan (2009) also observed that intermittent heating mode could reduce colour degradation whereas continuous heating caused much severe colour degradation (Chandan et al., 2014; Zhu and Pan, 2009).

Table 2.11 Comparison of quality attributes of food products by hot air drying and drying with intermittent IR radiation.

Product	Mode of Drying with Intermittency, ∝	Drying Conditions	Drying Time (min)	% Reduction in Drying Time (compared with hot air)	Browning Index (BI)	Colour Degradation (∆E)	References
Potato	Hot Air ∝ = 1	80°C 1.4 m/s	345	-	42.53 ± 1.11	-	Chua and Chou, 2005; Kalathur and Kurumanchi, 2010
	IR ∝= 1		208	39.71	38.77 ± 0.62	10.4 ± 0.83	
	IR ∝ = 0.25		174	49.57		8.6 ± 0.68	
	IR ∝ = 0.50		119	65.51		11.8 ± 0.94	
	IR ∝ = 0.60		115	66.67		12.1 ± 0.97	
	Combined hot air and IR		195	43.48	32.48 ± 0.44	-	
Carrot	Hot Air ∝ = 1	80°C 1.4 m/s	345	-	107.49 ± 1.12	-	Chua and Chou, 2005; Kalathur and Kurumanchi, 2010
	IR ∝= 1		300	13.04	106.07 ± 0.85	10.0 ± 0.80	
	IR ∝ = 0.25		220	36.23		3.2 ± 0.25	
	IR ∝ = 0.50		150	56.52		6.2 ± 0.49	
	IR ∝ = 0.60		145	57.97		13.1 ± 1.00	
	Combined hot air and IR		195	43.48	100.53 ± 0.72	-	

Product	Drying Mode	Drying Conditions	Drying Time (min)	% Reduction in Drying Time (compared with hot air)	Browning Index (BI)	Colour Degradation (ΔE)	References
Onion	Hot Air	60°C 2 m/s	340	-	18.99	26.60	Praveen et al., 2005
	IR		280	17.65	15.85	30.62	
	Combined hot air and IR		220	35.29	13.74	20.63	
Blueberry	Hot air	60 °C 4 m/s	960	-	✓ IR drying produced much firmer-texture product with much increased drying efficiency compared to conventional air drying ✓ Effective moisture diffusivity and activation energy were higher at IR drying compared to conventional air drying		Shi et al., 2008
	Infrared, IR=4000 W/m^2	60 °C	540	44.00			
		70 °C	210	78.00			
		80 °C	120	88.00			
		90 °C	90	91.00			

Mean values ± standard deviation (n =3 replications) within the same column are not significantly different (p > 0.05).

2.3.3 Optimization of intermittent drying

During intermittent drying, different types of intermittency may affect product quality and energy efficiency. Therefore, the intermittency should be selected based on the heat and mass transfer mechanisms involved during drying and properties of the drying product. Besides, the best combination of drying temperature, airflow rate and chamber pressure should be carefully determined and designed to ensure optimum production time and energy cost for the intermittent drying process (Chin and Law, 2010; Putranto et al., 2011). Several researchers have investigated different combination of operating variables on optimization of intermittent drying by using RSM as shown in Table 2.12.

However, optimization of intermittent drying is considered as a challenging task because of its extensive manipulation on many decision variables in achieving the desired multi-objective functions. In consequence, a suitable process tool such as design of experiment (DOE) or fractional factorial design must be chosen to develop algorithms that formulate and solve the optimization problem (Ong and Law, 2010b). The most popular experimental design used is central composite design (CCD) and Box-Behnken design (BBD) in Response Surface Methodology (RSM) to get appropriate quantitative data from experiments to determine and solve multivariate optimization problem. The first step in RSM is to determine operating variables that have significant impacts on interested response variables. In order to visualize the relationship between the operating and response variables, three-dimension response surface plots and contour plots are developed. At the end, optimization is performed based on the curvatures to obtain the highest product quality (maximize the response variables) at minimum operating cost (minimize the operating variables) (Montgomery, 2001).

Table 2.12 Selected works on optimization of intermittent drying by using response surface methodology (RSM).

Product	Experimental design and operating variables	Response variables	Optimized conditions and product quality
Red Currant (Sumic et al., 2016)	➢ 3-level, 3-factor fractional design ➢ Variables: X1 = Temperature (48-78°C) X2 = Pressure (30-330mbar) X3 = Drying time (8-16hr)	➢ Y1 = Total phenols content ➢ Y2 = Total flavonoids content ➢ Y3 = Monomeric anthocyanins content ➢ Y4 = Ascorbic acid content ➢ Y5 = DPPH content	➢ Optimum settings: X1 = 70.2°C, X2 = 39mbar, X3 = 8hr ➢ Vacuum dried samples better than conventionally dried sample and with good physiochemical properties
Olive leaves (Erbay and Icier, 2009)	➢ 3-level, 3-factor fractional design ➢ Variables: X1 = Temperature (40-60°C) X2 = Air velocity (0.5-1.5m/s) X3 = Drying time (240-480min)	➢ Y1 = Total phenolic content ➢ Y2 = Antioxidant activity ➢ Y3 = Final moisture content ➢ Y4 = Exergetic efficiency	➢ Optimum conditions: Minimum Y1 and Y2 with maximum X3 ➢ Optimum settings: X1 = 51.16°C, X2 = 1.01m/s, X3 = 298.68min
Pomegranate juice (Thirugnanasambandham and Sivakumar, 2015)	➢ 3-level, 3-factor fractional design ➢ Variables: X1 = Temperature (130-160°C) X2 = Feed flowrate (4-8rpm) X3 = Aspirator rate (50-100%)	➢ Y1 = Moisture content ➢ Y2 = Hygroscopicity ➢ Y3 = Powder yield	➢ Lower moisture content, better hydroscopicity and powder yield ➢ Optimum settings: X1 = 130°C, X2 = 6rpm, X3 = 100%
Okara (Wang et al., 2016)	➢ 3-level, 3-factor fractional design ➢ Variables: X1 = Temperature (50-70°C) X2 = Air velocity (1.3-2.3m/s) X3 = Sample loading density (3-4 kg/m^2)	➢ Y1 = Drying rate ➢ Y2 = Colour ➢ Y3=Trypsin inhibitor activity ➢ Y4 = Soy isoflavone content ➢ Y5 = Antioxidant activity	➢ Optimum settings: X1 = 70°C, X2 = 2.3m/s, X3 = 3 kg/m^2 ➢ Higher X1, higher X2 and lower X3 yielded to higher Y1

2.4 Transport Properties

2.4.1 Drying characteristics

Movement of moisture within a solid during drying is a complex process with various diffusion mechanisms such as molecular diffusion, capillary diffusion, surface diffusion or combinations of the above. All these drying characteristics can be studied through a drying curve by plotting the free moisture content (X) against drying time. Drying rate is the rate of change of moisture content over a certain time interval and this can be obtained by plotting drying rate versus moisture content.

A typical drying rate curve would consist of three major drying periods namely the constant rate period and the two distinctive falling rate periods as shown in Figure 2.10. At the initial transient period, solid is at a lower temperature (or vice versa) than that in drying chamber in the beginning of drying process. As drying continues solid surface temperature increases (or vice versa) and approaches equilibrium temperature in drying chamber. This initial period is usually short.

Then, the drying process continues to constant drying period that is governed fully by the rates of external heat and mass transfer since a film of free water is always available at the evaporating surface. It is also independent of internal moisture movement. Drying rate during this period is constant and the drying curve shows a straight horizontal line. Many foods and agricultural products, however, do not display the constant rate period at all because a layer of water is absent on the surface. In this case, falling rate prevails since internal heat and mass transfer determine the rate at which water becomes available at the exposed evaporating surface (Mujumdar and Devahastin, 2008).

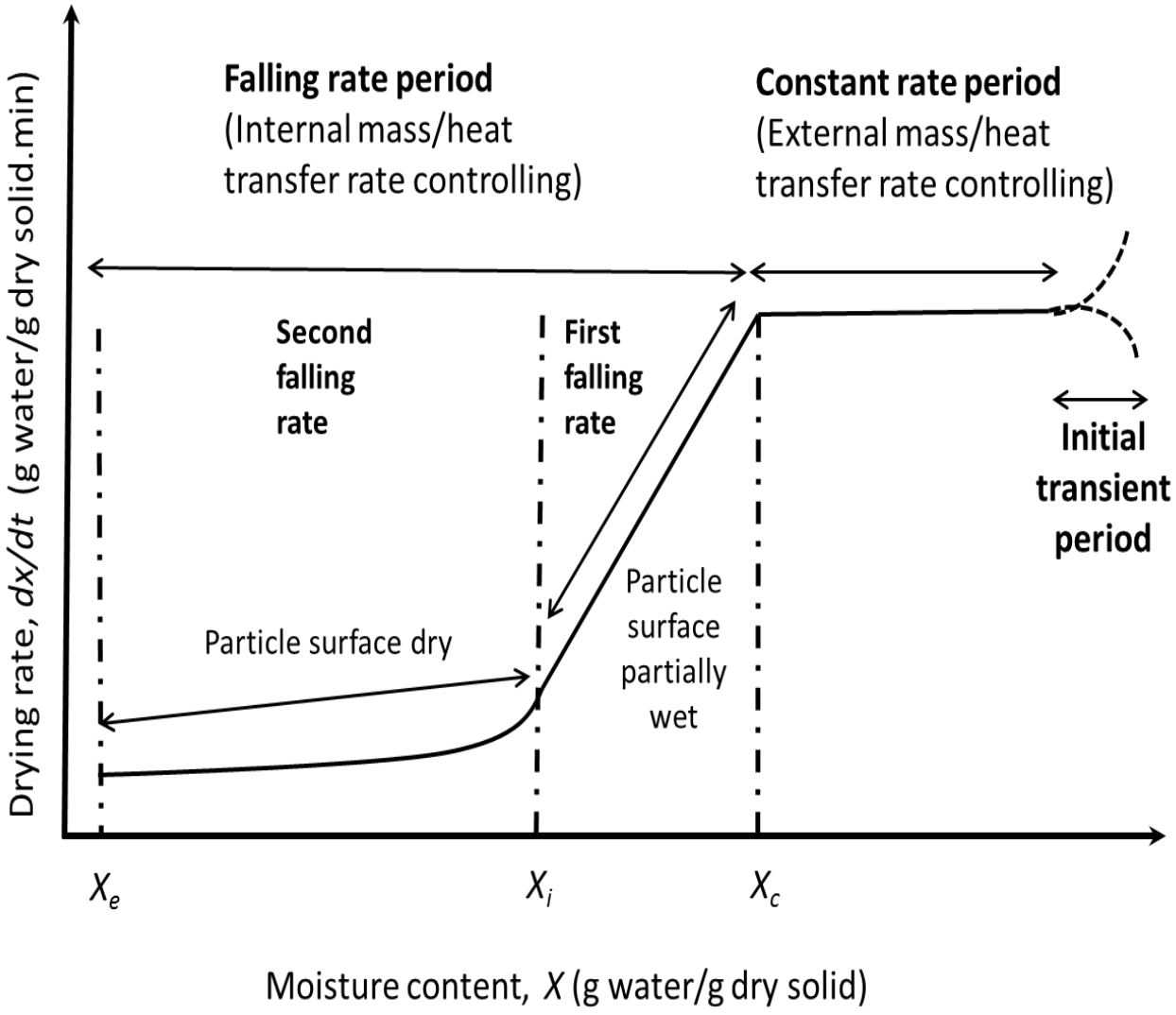


Figure 2.10 Drying rate curve

At the falling rate period, drying rate begins to fall with further decrease in moisture content since moisture cannot migrate to the surface easily due to internal transport limitations. The mechanism underlying this phenomenon depends on both the material and drying conditions. The drying surface becomes first partially unsaturated and then totally unsaturated until it reaches the equilibrium moisture content (Mujumdar and Devahastin, 2008).

2.4.2 Moisture diffusivity

According to Mujumdar and Devahastin (2008), moisture migration process during drying is complex and often involves one or more transport mechanisms such as vapour diffusion, liquid diffusion, Knudsen diffusion, surface diffusion and hydrostatic pressure differences. The flow of moisture within a material is usually expressed as moisture diffusivity in drying. Moisture is transferred mainly by molecular diffusion in the falling rate period of drying. Generally, Fick's second law of diffusion in equation [2.1] is used.

$$\frac{\partial X}{\partial t} = \nabla.(D_{eff}\,\nabla X) \qquad [2.1]$$

where X is the moisture content (kg water/kg dry matter), t is the drying time (s), ∇ is a gradient and D_{eff} is the effective moisture diffusivity (m^2/s).

Generally, moisture diffusivity in food materials is lower than that in non-food materials (Geankoplis, 1993). This is because of the complicated bioplolymer structure and strong binding of water molecules to cell wall in food. Extensive reviews with regards to the methods of experimental determination and compilation of moisture diffusivity data for various foodstuffs have been published in various literatures (Zogzas et al.,1994; Zogzas et al., 1996a). Normally, diffusivity values fall between 10^{-13} and 10^{-6} m^2/s while most of the diffusivity values in food material fall between 10^{-12} and 10^{-8} m^2/s (Zogzas et al., 1996b).

Overall, it can be concluded that effective diffusivity increases with temperature but varying trends are observed with respect to moisture content. At high temperature, the water molecules are loosely bound to the food matrix, thus requiring less energy to remove than at lower temperature (Xiong et al., 1992). In contrast, the dependency on moisture content depends greatly on the structure of food product. It was reported that for low porosity materials, the value of D_{eff} is very close to liquid diffusivity; while for granular and porous

materials moisture is transported mainly by vapour diffusion through the void space (Karathanos et al., 1990).

2.4.3 Thermal diffusivity

Thermal properties of food products, including specific heat capacity and thermal conductivity that describe the ability of material to accumulate and to transport heat, are the main intrinsic properties in modelling and evaluation of food processing operations that involve heat transfer, energy utilization and product quality (Lewicki and Jakubczyk, 2004). Normally, Fourier's heat conduction model in equation [2.2] is used to describe heat transfer within a food product during drying and effective thermal diffusivity (α_{eff}) is determined as shown in equation [2.3].

$$\frac{\partial T}{\partial t} = \nabla.(\alpha_{eff}\,\nabla T) \qquad [2.2]$$

$$\alpha_{eff} = \frac{k}{\rho C_p} \qquad [2.3]$$

where k is the thermal conductivity (W/m.K), ρ is the density (kg/m^3), C_p is the specific heat capacity (kJ/kg.K), T is the temperature (K) and t is the time (s).

Thermal properties depend very much on temperature and also composition of individual constituents in food such as water, protein, fat and carbohydrate. By knowing the composition of these constituents, thermal properties of a food material can be determined empirically. Various equations are available in literatures that relate thermal properties of these constituents with temperature. In most cases, thermal conductivity and heat capacity of food material would increase with increasing moisture content above freezing temperature. This is because water has a much higher specific heat and thermal conductivity than the other major food constituents such as protein, fat and carbohydrates. Thus, moisture content can greatly influence the thermal properties of foods. (Kouchakzadeh and Tavakoli, 2009; Rahman et al., 1997; Sweat, 1995).

2.5 Physiochemical Properties

2.5.1 Colour

One major issue concerning edible bird nest processing is severe colour change in dried EBN. After cleaning and drying, the bird nest turns yellowish which is undesirable to consumers. EBN manufacturers normally overcome the problem by applying bleaching agents which are chemical based and hazardous to health (GuangZhou News, 2011; ZheJiang News, 2011). The severe colour change such as browning (yellowish) of EBN is due to improper processing conditions such as high drying temperature and long processing duration.

EBN are usually white but some are found in caves with dull brownish or orange red colours. In the market, red blood nests are more expensive than white EBN, which can be sold for up to US$10,000 per kilogram, due to their rarity and have been traditionally claimed to have better health benefits. It was thought that red EBN are produced from swiftlet's blood that mixed with the saliva or due to the special type of food that swiftlet consumed. However, EBN that are too red could be due to the result of dye additives and the red colour may dissolve when cooked (Ma and Liu 2012). Others believe that the caves contain specific minerals and iron which turn the EBN to red colour (Shaw et al., 2013). Different explanations have been suggested but there is lack of scientific evidence to support these claims.

In fact, the observed red colour is caused by nitrifying bacteria that present in the bird's nests which reacts with ammonia vapours from the decaying guano. Having figured this out, some traders began treating ordinary white nests with ammonia to produce the more expensive red nests. Hence, the severe colour change is also due to the high contents of nitrite and nitrate which has prompted the Chinese

government to ban the import of Malaysian EBN in 2011 due to the high nitrite content (Food Quality News, 2011; Xinhuanet, 2012).

Commission Internationale de I'Eclairage (CIE) colour parameters is normally used to describe visual colour deterioration for quality control in food products. The colour brightness CIE *L**measures the whiteness and ranges from black (0) to white (100). CIE *a** measures red when positive and green when negative whereas CIE *b** measures yellow when positive and blue when negative. Parameter chroma indicates the degree of colour saturation and is proportional to the strength of the colour while hue angle is used to represent the purity of brown colour especially when enzymatic and non-enzymatic browning takes place (Elcin and Belma, 2009).

2.5.2 Moisture reabsorption ability

In order to quantify the capacity of dried EBN to reabsorb moisture, three indexes were used namely dry basis holding capacity (DHC) index, water absorption capacity (WAC) index and moisture reabsorption ability (RA). Rehydration experiments are usually performed by immersing a weighed amount of dried samples into hot water at 50°C for a period of time i.e. 50 minutes. At every 10-minute interval, the samples are drained over a mesh for 30 s and quickly blotted dry with paper towels to eliminate the surface water and then reweigh until there is no further change in weight (Giri and Prasad, 2007).

After rehydration, the mass of rehydrated sample is weighed and the expansion coefficient of the food sample is calculated. The dry basis holding capacity (DHC) index or also known as expansion coefficient (range of $0 \leqslant WAC \leqslant 1$), is calculated as a measurement of the ability of the material to retain soluble solids/water sorption during rehydration, to provide information on the extent of tissue damage (Cunningham et al., 2008). Water absorption capacity (WAC) index

(range of $0 \leqslant WAC \leqslant 1$) is the ability of the matrix to absorb water with respect to water loss during drying (Prabhanjan et al., 1995). The rehydration ability (RA) index measures the ability of the dried product to rehydrate as affected by damage on tissue caused by drying and rehydration processes (Moreira et al., 2008).

2.5.3 Shrinkage

Shrinkage during drying determines the rate of drying and the quality of the dried product. Researchers have expressed shrinkage as a function of dimensional changes of the samples measured using vernier or digital callipers (Karathanos et al., 1996). Generally, it is expressed in terms of the apparent volume as shown in equation [2.4]. The volume can be measured by applying Archimedes principle or by a number of displacement techniques (Yan et al., 2008).

$$S = \frac{V_d}{V_o} \qquad [2.4]$$

where V_d is the apparent volume of the sample after drying (m^3) and V_o is the apparent volume of the raw sample (m^3).

Shrinkage occurs because food polymers cannot support its weight and hence collapse in the absence of moisture. Recently, there have been studies to describe the shrinkage behaviour of various fruits and vegetables using prediction models. Lozano, Rotstein and Urbician (1983) reported a general correlation for predicting shrinkage of fruits and vegetables with changing moisture content. McMinn and Magee (1997) reported a linear correlation for shrinkage with moisture content and air temperature during drying of cylindrical samples of potato in a tunnel dryer. Hernandez et al. (2000) and Park (1998) proposed a linear relationship for shrinkage of foods as a function of moisture content. Thus, it is evident that the physical properties change with reduction of moisture content. Furthermore, the

change in physical dimensions is product specific and varies according to drying conditions.

2.5.4 Storage stability

During drying, limiting accessibility of active water in dried product (with moisture content less than 15-20% or water activity below 0.70) may diminish undesirable biochemical and chemical reactions during storage, thus prolong shelf life of the dried food products (Mujumdar and Law, 2010). It was found that the colour of dried EBN products would degrade with storage period. However, the degradation rate may vary with drying methods and storage conditions (eg. storage temperature, storage relative humidity and air composition). Thermal effect during drying may destruct heat labile components in food product during drying and hence affects its stability in subsequent storage. Hence, the stability of chemical, biochemical, physical properties of the food materials throughout storage must be determined by observing the thermal treatment history, which further influences the shelf life and quality of the final product (Paydar et al., 2013; Pragati et al., 2003).

2.6 Biochemical Properties

2.6.1 Nitrite

In 2011, analysis conducted by China Mainland authority and local studies found that 200 ppm of nitrite content was found in various bird's nests available in the China market, particularly the blood-red bird's nest (Centre for Food Safety Hong Kong, 2011). Hence, the regional authority has strictly controlled the nitrite concentration in all EBN products to prevent food toxicity since nitrite can react with secondary amines in food products or in the digestive system to form nitrosoamines which is a class of carcinogenic compounds (Department of Standards Malaysia, 2012).

In general, formation of nitrite and nitrate is caused by a process called nitrification. Nitrification is the conversion of ammonia to nitrate which is mainly performed by soil-living bacteria and other nitrifying bacteria as shown in Figure 2.11. Nitrobacteria uses energy from oxidation of nitrite to nitrate to fulfil their energy needs. Nitrobacteria fix carbon dioxide via "Calvin cycle" for their carbon requirements (Grundmann et al., 2000). According to Holt et al. (1993), nitrifying bacteria are photosensitive especially to blur and ultraviolet light.

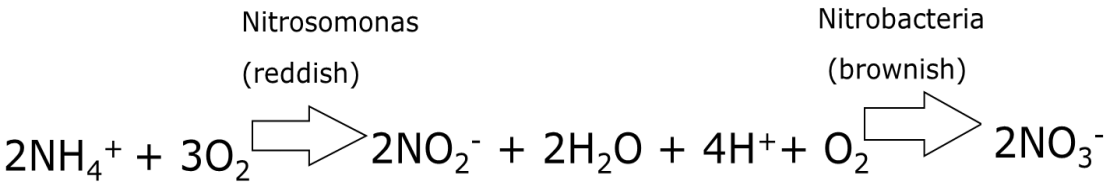


Figure 2.11 Chemical reaction in nitrification (Paydar et al., 2013).

Chan (2013) presented the result of proteomic analysis on EBN and showed that a protein was identified as periplasmic nitrate reductase originated from nitrogen fixing bacteria (Nitrobacteria). Most of the EBN producing regions in Asia, including Indonesia and Malaysia, are located within isolated islands. The plants that usually grow on these islands are suffering from nitrogen deficiency. Therefore, the plants would develop symbiosis with the nitrogen fixing bacteria to provide space (i.e. root nodules) for the growth of nitrogen fixing bacteria in exchange of the nitrate as nutrients. Possibly, the nitrogen fixing bacteria managed to reach the swiftlet oral cavity through the swiftlet's insect preys or directly from the plants or soil. Then, nitrogen fixing bacteria could be released through salivary secretion and eventually contaminated EBN (Chan, 2013).

Paydar et al. (2013) hypothesized that sources of nitrite and nitrate could have been derived from ammonia through anaerobic fermentation by nitrobacteria. Nitrobacteria are a genus of mostly rod-shaped, gram-negative and

chemoautotrophic bacteria. It is mostly found in soil, freshwater and on building surfaces, especially in areas that contains high levels of nitrogen compounds. Grundmann et al. (2000) stated that Nitrobacteria seem to grow optimally at 38°C and at pH 7.9, while Holt et al. (1993) stated that Nitrobacteria seem to grow optimally at 28°C and at pH 7.6 - 7.8 and will die at temperature exceeding 49°C or below 0°C.

Basically, nitrate (NO_3^-) and nitrite (NO_2^-) are naturally occurring ions that are ubiquitous in the environment. Both are products from the oxidation of nitrogen by microorganisms in plants, soil or water and to a lesser extent, by electrical discharges such as lightning. Nitrate is the more stable form of oxidized nitrogen but still can be reduced by microbial action to nitrite. Aromatic amino acids such as tyrosine, phenylalanine and tryptophan in EBN possess phenyl rings that could react with nitric / nitrous acid, which are derived from hydrochloric acid and sodium nitrate reaction or bacterial fermentation of bird droppings, to form yellow EBN through formation of aryl-C-N and NO_2 side groups. Sodium or calcium in natural habitat when react with water will form sodium or calcium hydroxide (base), which cause pH increment in the presence of activated aromatic rings that may further promote severe colour changing process (brown or red) (Paydar et al., 2013).

2.6.1.1 Correlation of nitrite content and colour change

Paydar et al. (2013) suggested that colour of EBN is associated with the prevalence of the nitrite and nitrate contents. White EBN contained low amounts of nitrite and nitrate. There is evidence that the foaming of EBN with sodium nitrite and hydrochloric acid increased the levels of nitrate and nitrate that may change the colour of white EBN. Enhancing nitrite level by adding sodium nitrite alone has no effect on white EBN, indicating that chemical reaction is necessary for the colour change (But et al., 2013).

Besides, another validation study to understand the nitrite production and accumulation process in EBN during cultivation was performed by Chan (2013) which showed that the addition of nitrate source on white edible bird's nest could turn the colour of EBN to yellow colour after 10 days and eventually became red after 20 days. Similarly, yellow EBN turned into red colour after 10-15 days of incubation with 1M potassium nitrate. No significant change in the colour of red, white and yellow EBN when sources of nitrate is absent (Chan, 2013).

On the other hand, nitrite instead of nitrate reagent such as 1M sodium nitrite was applied on EBN with different colours. Again, white EBN eventually turned into red colour in a faster manner. The white EBN took only 5 days to become yellow and 10 days to become red; while yellow EBN took less than 10 days to become red colour. There were no significant changes in the appearance (colour) of red, white and yellow EBN when nitrite sources are absent (Chan, 2013). The effect of sodium tungstate on the colour change of EBN was also examined. Sodium tungstate, a specific inhibitor for nitrate reductase, was applied onto EBN during cultivation. The white and yellow edible bird's nest eventually turned into red colour after 20 days and 15 days, respectively, with nitrate source. The absorbance increased as the content of nitrite increased. In conclusion, there is a strong correlation between nitrite content and the colour of EBN.

2.6.1.2 Effects of nitrite

The high level of nitrite found in EBN has raised public concern, casting doubt whether these EBN are truly "edible". Sodium nitrite has been used as a food preservative, anti-microbial agent in the food processing industry to protect against microorganisms growth (especially useful for killing *Bacillus botulinum)*, colour fixative and flavouring many food products. However, its level is strictly controlled to prevent food toxicity because nitrite can react with secondary

amines in food products or in the digestive system to form nitrosoamines which is a class of carcinogenic compounds (DSM, 2011).

Furthermore, nitrate can be readily converted into nitrite by microbial reduction and about 20% of nitrate may be converted to nitrite in the mouth by action of saliva and bacteria while the remaining can be converted in the stomach (Kamaruddin, 2012). Nitrite can also oxidize haemoglobin in blood and make it unable to carry oxygen to the body tissues, thus the victims may develop blue or purple colouration in the lip and skin which is known as methaemoglobinaemia (Orgeron et al., 1957). Pregnant women who take excessive amount of nitrite are therefore exposed to high risk of having pre-mature baby due to lack of oxygen in the blood system (DSM, 2011).

Normally, nitrate presents as sodium nitrate, which is a more stable form but tend to be oxidized to nitrite through bacterial activity and oxygen. World Health Organization (WHO) has limited the allowable daily intake (ADI) of nitrate and nitrite to maximum of 3.7 mg and 0.07 mg per kg body weight, respectively. For example, the allowable daily intake for a 60 kg human body is 222 mg of nitrate and 4.2 mg of nitrite per day. Nitrite is naturally formed by through oxidation to form sodium nitrite ($NaNO_3$). Thus, it is impossible to keep EBN nitrite to 0 ppm. WHO Standard at 30 ppm nitrite level led to the introduction of MS 2334:2010 Edible Bird's Nest (EBN) Specification and is also in line with the Food Regulations 1985 (AQSIQ, 2011) as shown in Appendix C (Table AI). Paydar et al. (2013) provided evidence that EBN from caves generally contained higher nitrite and nitrate levels compared to those from swiftlet houses. This is because most operators clean the swiftlet houses frequently by removing the bird soil which could contaminate EBN.

2.6.2 Sialic acid

Sialic acid is a family of nine-carbon acidic monosaccharides that occur naturally in the soluble proteins. N-acetylneuraminic acid (NANA) is one of the most predominant sialic acid occurring in nature (Shaw et al., 2001). Sialic acid is the major substitute of carbohydrate compound in EBN (Chan, 2006).

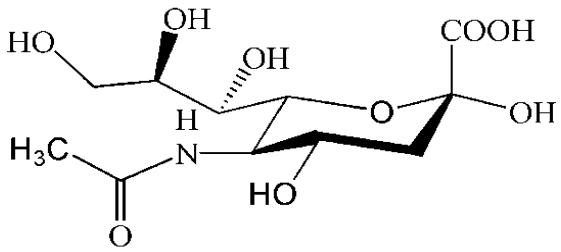


Figure 2.12 Molecular structure of N-acetylneuraminic acid (NANA).

EBN can inhibit influenza virus infection, which is mediated by the NANA residues of sialyl-sugar chains present in the EBN (Guo et al., 2006). Wang and Brand-Miller (2003) suggested that the exogenous source of sialic acid plays a role in brain development and learning ability. In Malaysia, sialic acid could be a potential criteria to be used for determining the price of EBN (Marni et al., 2014).

Despite its role of acting as a "decoy" for invading pathogens, sialic acid is also known as an agent necessary for mediating gaglioside distribution and structure in the brain. It was found that an exogenous source of sialic acid may contribute to neurological and intellectual advantages in infants (Chan, 2006). Guo et al. (2006) also claimed that sialic acid residues in EBN are involved in biologically important ligand-receptor interactions such as specific cell-to-cell, pathogen-to-cell or drug-to-cell interactions (Chan, 2006).

Interestingly, there are some differences between the inhibitory activity of EBN harvested from caves and from bird's house. This could be due to the differences

of the living environment and the destruction of O-acetyl sialci acid in EBN when collected from caves. The results suggested that EBN is a safe and natural source for prevention of influenza viruses in vitro (Guo et al., 2006).

2.6.3 Antioxidant

An antioxidant is defined as a substance when present at low concentrations compared with those of an oxidise substrate, significantly retards or delays a pro-oxidant initiated oxidation of the substrate. Antioxidant plays an important role at different steps in the oxidation sequence. The primary or chain-breaking antioxidant reacts with lipid radicals to yield more stable products and these antioxidants are known as free-radical interceptors. The secondary or preventive antioxidants decrease the rate of chain initiation by several different types of mechanisms which include metal inactivators, hydroperoxide decomposer, oxygen scavengers and synergists (Eskin and Pryzbylski, 2001). Natural antoxidants can be divided into two main groups which are plant and protein antioxidants.

2.6.3.1 Plant antioxidants

Common example of plant foods antioxidant are such as from carrot, turmeric, tea leaf, walnut, green vegetables, tomato and citrus fruits. The total antioxidant capacity of walnut kernel ranked at the top of the scale among various fruits and vegetables. Among the common plant foods consumed worldwide, walnut is ranked second only to rose hips in their antioxidant activity, as determined by the ferric-reducing antioxidant power assay (Halvorsen et al., 2002). Polyphenols are the major plant bioactive compounds with antioxidant activity. Plant foods also contain an immense variety of biologically-active, non-nutritive compounds that contribute to colour, flavour and other characteristics. These phytochemicals such as phytoestrogens, carotenoids and flavonoids have been increasingly linked to

the reducing risk of chronic disease such as cancer, osteoporosis and coronary heart disease (Johnson and Williamson, 2003).

2.6.3.2 Protein antioxidants

Various studies have reported the antioxidant potential of biologically active peptides from protein hydrolysates such as soy protein (Moure et al., 2006), wheat protein (Zhu et al., 2006), milk casein (Kunio et al., 2000), animal mucous and fish protein (Wu et al., 2003) as shown in Table 2.13. According to Jun et al. (2010), the antioxidant activities of peptide are closely related to their amino acid constituents and their sequence.

Table 2.13 Comparison of antioxidant activity by FRAP assay by on different types of plants and protein sources.

Antioxidant sources	Antioxidant content (mg TEAC/g dw)	Reference
Edible bird's nest	86.46 ± 0.21	Guo et al., 2006
Ginger	19.37 ± 0.81	An et al., 2016
Chokeberry	39.00 ± 13.5	Samoticha et al., 2016
Tomato	14.24 ± 5.32	Gumusay et al., 2015
Apple	106.3 ± 15.46	Vega-Galvez et al., 2012
Soy protein	16.27 ± 0.43	Durazzo et al., 2015
Cow milk	51.18 ± 2.66	Durazzo et al., 2015

EBN extract gives relatively high antioxidant capacity under certain extraction method. Antioxidant activities in EBN extract could be due to the presence of amino acids such as cysteine, methionine, histidine, tryptophan and lysine that have been proven contain stronger antioxidant activity (Guo et al., 2006; Noraini, 2012; Nurfatin, 2014). Two essential amino acids, lysine and tryptophan, are not usually present in most plant proteins. A plausible mechanism of antioxidant activity for EBN may be due to the hydroxyl and carboxyl groups in the amino acids. Active oxygen plays an important physiological role but may exert toxic effects as well which could contribute to a causative role in heart disease, cancer and aging (Guo et al., 2006).

CHAPTER 3 METHODOLOGY

3.1 Sample Preparation

Fresh farm EBN was obtained from a bird nest processing plant (LiZhi Trading) in Sungai Pelek, Selangor, Malaysia. EBN with similar size (L=8.00±2.15 cm x W=4.00±1.98 cm x H=0.52±0.03 cm) and colour (L^* = 57.0±0.24, a^* = 0.75±0.5 and b^* = 8.50±0.5) were selected for all experiments. The feathers in fresh EBN were removed by workers manually with tweezers. In each experiment, EBN was taken directly from plant after the cleaning process in order to avoid loss of moisture content. In total three pieces of cleaned EBN (weighed about 14g – 20g each) were placed on a drying tray in each experiment. The average initial moisture content of fresh farm EBN was approximately 140% - 160% (dry basis) and the targeted final moisture content after drying was at 10% - 12% (dry basis).

3.2 Drying Experiment

3.2.1 Dryer Description

3.2.1.1 IR-UVC assisted dryer

An IR-UVC assisted dryer was fabricated and supplied locally by I-Lab Sdn. Bhd. (Selangor, Malaysia). The dryer was made from stainless steel and the schematic diagram of the dryer is shown in Figure 3.1. Actual diagrams of outer and inner compartment of dryer are shown in Appendix A. The dryer has an outer dimension 710 mm x 655 mm x 800 mm (L x W x H) and an inner compartment 605 mm x 603 mm x 340 mm (L x W x H). Double perspex walls were installed in the dryer (wall thickness = 5mm) and the distance between the first and second layer of perspex wall was 10mm.

Four infrared (IR) lamps (230 V, 1000 W, maximum overall wavelength 357.5 nm) (model HW-1000, Yanming Lighting, Shanghai, China) of diameter 150 mm were

installed inside the drying chamber of the dryer (two at the top and two at the bottom). Four ultraviolet C (UVC) lights (23.6 V, 4 W, short-wave UVC radiation with a maximum peak at 253.7 nm) (model TUV 4W FAM, Yanming Lighting, Shanghai, China) of diameter 16 mm were also installed on the side walls of drying chamber. The lamps were connected to a regulating transformer, which enabled the power supplied to the lamp to be varied from 0 to 240 V. When the average power was kept at 240 W, an average radioactive heat flux of 0.23 W/m^2 was obtained for the experiments. Four self-rotating perforated sample holder made from steel wire mesh with diameter of 152 mm were installed in the drying chamber and revolved round a central gear.

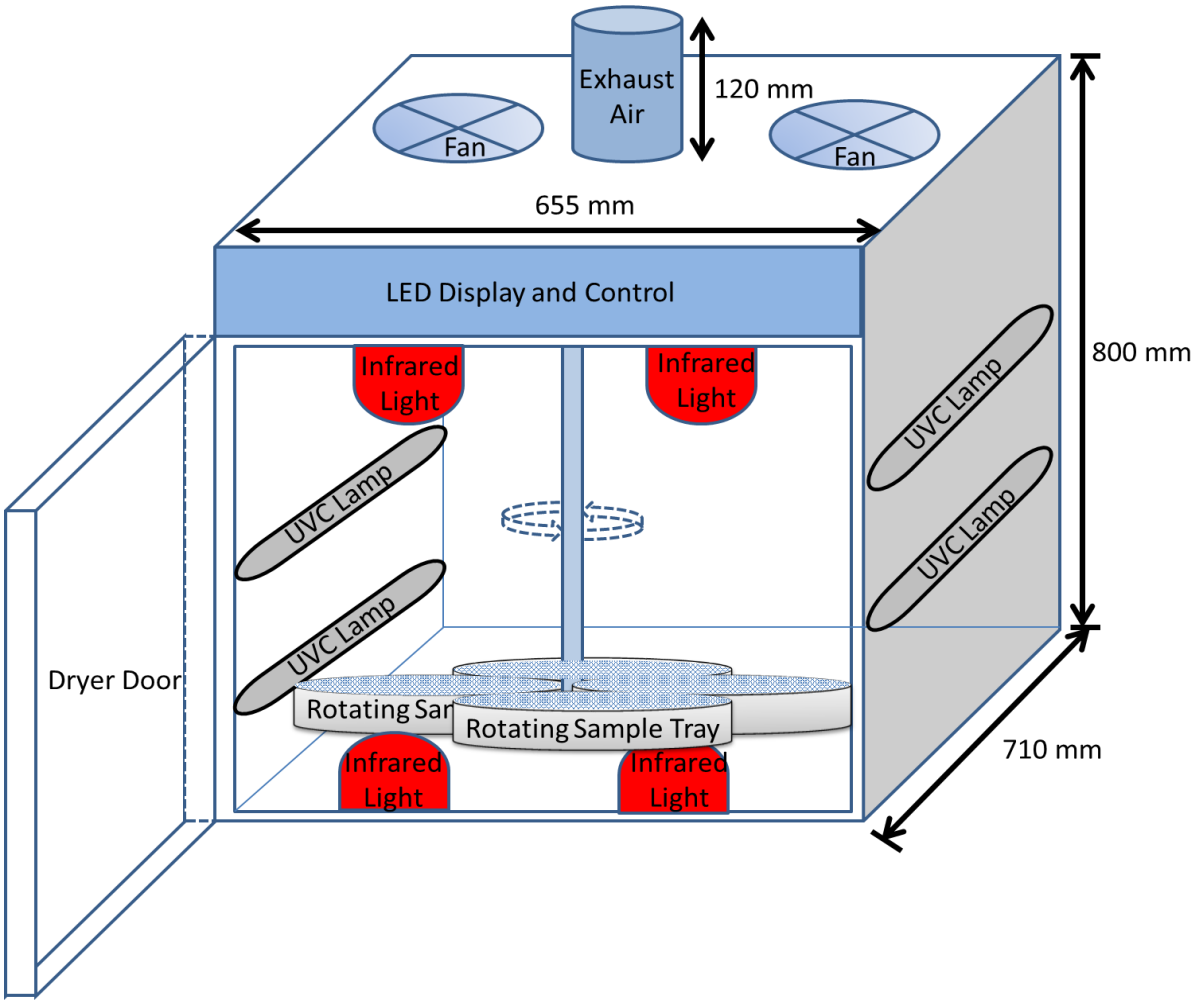


Figure 3.1 The schematic diagram of IR-UVC assisted dryer.

Temperature could be controlled within a range of 30°C-70°C. The speed of turning table was set at 3.4 ± 0.5 m/s. Air velocity was fixed at 4.6 ± 0.2 m/s in the drying chamber throughout drying. The dehumidified air with desired temperature and relative humidity conditions was regulated using three Proportional Integral Derivative (PID) controllers. EBN was placed on a bird's nest moulding tray with size of 12cm x 7cm x 5cm, perpendicular to the air flow. The drying chamber was operated at drying temperature of 40°C and relative humidity (RH) of 16.5 % when the heater was turned on, whereas the drying temperature was 25°C and 26.7 % RH when the heater was turned off. The dryer could be operated in two operating modes namely continuous and intermittent mode. Drying intermittencies, α used were 0.2, 0.33 and 0.67 with tempering periods of 4, 2 and 1 hour, respectively, as shown in Table 3.1. Intermittency is calculated based on equation [3.1] (Chandan et al., 2014).

$$\text{Intermittency, } \alpha = \frac{T_{ON}}{T_{ON} + T_{OFF}} \qquad [3.1]$$

where T_{ON} and T_{OFF} are "on" and "off" periods of IR and UVC lamps, respectively.

Table 3.1 Operating conditions in the IR-UVC assisted dryer.

External Heater	Drying Temperature (°C)	Relative Humidity, RH (%)	Drying Period, t_{drying} (hr)	Tempering Period, $t_{tempering}$ (hr)	Intermittency, α
On	40	16.5	Continuous	-	1
			2	1	0.67
			1	2	0.33
			1	4	0.20
Off	25	26.7	Continuous	-	1
			2	1	0.67
			1	2	0.33
			1	4	0.20

3.2.1.2 Hot air dryer

Hot air drying was performed using a laboratory-scale air circulation oven (Memmert, Schwabach, Germany) with air temperature of 60°C, 70°C and 80°C (accuracy of ±0.5ºC) and relative humidity of 10.1%, 8.3% and 2.2% RH (accuracy of ±0.05%), respectively. The air circulation was set at a velocity of 1.0 m/s. EBN was placed on a bird's nest moulding tray with size of 12 cm x 7 cm x 5cm perpendicular to the air flow.

3.2.1.3 Fan assisted drying

The fresh EBN was dried using a standing fan (model F-MT405, Panasonic, Selangor, Malaysia) with air delivery capacity of 47.5±0.45 m^3/min in a closed room at air temperature of 27°C (accuracy of ±0.5°C) and relative humidity of 39.7 % RH. The air circulation was set at a velocity of 5.0 m/s. EBN was placed on a moulding tray with size of 12 cm x 7 cm x 5cm parallel to the air flow. The total drying time to achieve equilibrium moisture content (EMC) was 19 hours for fan assisted drying. Fan assisted drying is the most common drying practice by EBN manufacturers.

3.2.2 Drying Procedure and Measurements

Fresh EBN samples were subjected to IR-UVC assisted drying, hot air drying and fan assisted drying. The operating conditions of the dryer in each experiment are as shown in Table 3.2. Weight loss of the sample was monitored using an analytical balance (JS303G, Mettler Toledo, Switzerland) and recorded periodically at 5-min intervals in the first 60-min and 30-min intervals and thereafter until a constant reading was obtained in three consecutive measurements. The moisture content of the sample at this stage was marked as the equilibrium moisture content (EMC). The bone-dry weight (d_w) was determined by drying the sample in an oven at 105°C for 24 hours.

Table 3.2 Operating temperature, relative humidity (RH) and air velocity used in dryers and various intermittencies, α are applied.

Drying System	Remarks	α*	T (°C)	RH (%)	v (m/s)
Fan Drying (FAN27)	Continuous	1.00	27	39.7	5.0
Hot air drying (HA80)	Continuous	1.00	80	8.3	4.6
Low Temperature Drying (LT25)	Continuous	1.00	25	30.2	4.6
Infrared Assisted Drying (IR25)	Continuous	1.00	25	29.5	4.6
UVC Assisted Drying (UVC25)	Continuous	1.00	25	29.3	4.6
IR-UVC Assisted Drying (IR-UVC40)	Continuous Intermittent	1.00 0.67* 0.33* 0.20*	40	16.5	4.6
IR-UVC Assisted Drying (IR-UVC25)	Continuous Intermittent	1.00 0.67* 0.33* 0.20*	25	27.5	4.6

*Intermittency, $\alpha = \frac{T_{ON}}{T_{ON}+T_{OFF}}$

where T_{ON} and T_{OFF} are the "on" and "off" periods respectively of the dryer.

Upon drying, the whole piece of dried EBN was crushed into smaller parts (0.2 cm) and analysed for water activity (Decagon, Pawkit, Pullman, WA). Temperature and relative humidity in the drying chamber were determined using a hygrometer attached to a datalogger (RS232, HygroFlex, Hauppauge, NJ), which was equipped with digital probes and HW3 software. The air flow was measured by placing an anemometer perpendicular to the air flow (LCA30VT, Airflow, Buckinghamshire, UK) with accuracy ±0.01 m/s. Besides, the temperature of each EBN sample was measured using a 0.3 mm diameter T-type thermocouples that were inserted into the centre of the sample.

3.3 Determination of Transport Properties

3.3.1 Drying kinetics

Moisture content (MC) of edible bird's nest was determined with reference to the bone-dry weight of the nests (determined by the oven drying method) using equation [3.2] (Hii et al., 2010). The final moisture content was targeted as 10% - 12% (dry basis) and the drying kinetics curves were plotted. For the purpose of graphical presentation, the moisture ratio was defined based on the normalised moisture content equation [3.3] (Hii et al., 2010).

$$\text{Moisture content on dry basis (MC)} = \frac{W_o - W_d}{W_d} \quad [3.2]$$

where MC is the moisture content of the sample (g water/g dry matter), w_o is the initial weight of the sample (g) and w_d is sample weight at time i (g).

$$\text{Moisture ratio (MR)} = \frac{X_i - X_e}{X_o - X_e} \quad [3.3]$$

where MR is the moisture ratio, X_i is the moisture content at time i (g water/g dry matter), X_e is the targeted (or equilibrium) moisture content (g water/g dry matter) and X_o is the initial content (g water/g dry matter). Fan assisted dried samples were used as a benchmark in product quality assessment only.

3.3.2 Effective moisture diffusivity

EBN is considered as an infinite slab because the thickness at the middle is different from both left and right sides of the bird's nest. The moisture diffusivity for an infinite slab was proposed by Crank (1975) equation [3.4] considering assumptions mentioned below (Pathare and Sharma, 2006; Sharma et al., 2005).

(1) Moisture is initially evenly distributed throughout the mass of a sample.

(2) Mass transfer is symmetric with respect to the centre.

(3) Moisture content on the sample surface simultaneously reaches equilibrium with the condition of surrounding air.

(4) Internal resistance of the sample is negligible compared to the resistance to the mass transfer at the surface.

(5) Mass transfer is by diffusion only.

Crank using Fick's second law proposed equation [3.4] for the effective moisture diffusivity for an infinite slab (Crank, 1975).

$$\mathrm{MR} = \frac{X_i - X_e}{X_o - X_e} = \frac{8}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{(2n-1)^2}\exp[-(2n-1)^2\pi^2\frac{D_{eff}\,t}{4L^2}] \quad [3.4]$$

where MR is the moisture ratio, X_i is the moisture content at time *i* (g water/g dry matter), X_e is the targeted moisture content (g water/g dry matter), X_o is the initial content (g water/g dry matter), *n* is the number of terms taken into consideration, *t* is the drying time, D_{eff} is the effective moisture diffusivity (m^2/s) and *L* is the thickness of the sample (m).

Neglecting higher terms and by taking only n = 1, equation [3.5] is used by several researchers (Babalis and Belessiotis, 2004; Pathare and Sharma, 2006; Vasic et al., 2014) in order to describe the diffusion.

$$MR = \frac{8}{\pi^2}\exp[-\pi^2\frac{D_{eff}\,t}{4L^2}] \quad [3.5]$$

In the above equation, constant thickness, *L* is assumed throughout the drying process. Equation [3.5] can be simplified to a linear relationship as shown in equation [3.6] (Aghbashlo et al., 2008; Babalis and Belessiotis, 2004).

$$Ln\,(\mathrm{MR}) = Ln\left(\frac{8}{\pi^2}\right) - (\pi^2\frac{D_{eff}\,t}{4L^2}) \quad [3.6]$$

Effective diffusivity can be obtained by plotting Ln (MR) versus time, *t*. From equation [3.6], a plot of Ln (MR) versus time gives a straight line with a slope of k_1 in equation [3.7].

$$D_{eff} = \frac{4k_1L^2}{\pi^2} \quad [3.7]$$

Effective moisture diffusivity, D_{eff} obtained were then expressed in terms of Arrhenius equation based on equation [3.8]:-

$$D_{eff} = D_O \exp(\frac{-E_a}{R_g T}) \quad [3.8]$$

where D_o is the pre-exponential factor of the Arrhenius equation (m^2/s), E_a is the activation energy (kJ/mol) and R is the universal gas constant (8.3143 kJ/mol.K).

3.3.3 Effective thermal diffusivity

The effective thermal diffusivity (α_{eff}) was estimated by taking sum of the fractional contributions of each pure component (moisture, protein, carbohydrate, ash and fibre) in dried EBN samples as described in section 3.5.1 (Chen, 2008; Marcone, 2005).

Table 3.3 Proximate composition of each pure component in dried EBN samples.

Pure components	Proximate composition (% dry matter)
Moisture	11.32 ± 0.26
Crude protein	66.90 ± 1.60
Crude fat	0.8 ± 0.10
Carbohydrate	25.40 ± 1.25
Ash	6.80 ± 0.01
Fibre	0.10 ± 0.01

Note: Each value is the mean ± standard deviation after three replications.

$$\alpha_{moisture} = 0.1317 + 6.2477\times10^{-4}\, T - 2.4022\times10^{-6}\, T^2 \quad [3.9]$$

$$\alpha_{protein} = 0.0068714 + 4.7578\times10^{-4}\, T - 1.4646\times10^{-6}\, T^2 \quad [3.10]$$

$$\alpha_{fat} = 0.0098777 + 1.2569\times10^{-4}\, T - 3.8286\times10^{-8}\, T^2 \quad [3.11]$$

$$\alpha_{carbohydrate} = 0.0080842 + 5.3052\times10^{-4}\, T - 2.3218\times10^{-6}\, T^2 \quad [3.12]$$

$$\alpha_{ash} = 0.12461 + 3.7321\times10^{-4}\, T - 1.2244\times10^{-6}\, T^2 \quad [3.13]$$

Overall α_{eff} was determined by taking into account the weight fraction (W = 0-1).

$$\alpha = \sum \alpha_i W_i \quad [3.14]$$

3.4 Physiochemical Properties Analyses

3.4.1 Colour analysis

The colour measurements were performed by using a colorimeter (AccuProbe, HH06, USA) in a room with controlled light. The instrument was calibrated before the measurement with a white ceramic plate. The EBN samples were scanned at three different locations (eg. P1, P2 and P3) for both sides as shown in Figure 3.2 below to determine the average Commission Internationale de I'Eclairage (CIE) *L*, a* and b** values.

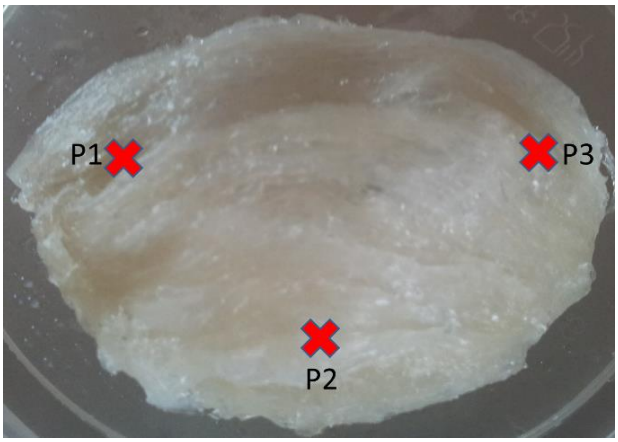


Figure 3.2 Three different locations in EBN for colour analysis.

The total colour change *(ΔE)* equation [3.15], chroma equation [3.16] and hue angle equation [3.17] (Dadali et al., 2007) were determined to describe the colour change during drying.

$$\Delta E = \sqrt{(L^*_0 - L^*_t)^2 + (a^*_0 - a^*_t)^2 + (b^*_0 - b^*_t)^2} \quad [3.15]$$

$$Chroma = (a^{*2}_t + b^{*2}_t)^{0.5} \quad [3.16]$$

$$Hue\ angle = tan^{-1}(\frac{b^*_t}{a^*_t}) \quad [3.17]$$

where L^*_o, a^*_o *and* b^*_o are the initial colour measurements of raw bird's nest samples and L^*_t, a^*_t and b^*_t are the colour measurements at a pre-specified time.

3.4.2 Moisture reabsorption ability and shrinkage

Dry basis holding capacity (DHC) index (also known as expansion coefficient) with a range of 0⩽WAC⩽1 was used as shown in equation [3.18] (Cunningham et al., 2008).

$$\mathrm{DHC} = \frac{m_{rh}}{m_{dh}} \quad [3.18]$$

where m_{rh} is the mass of rehydrated sample (g) and m_{dh} is the mass of the dry sample taken for rehydration testing (g).

Water absorption capacity (WAC) index with a range of 0⩽WAC⩽1was used to determine the ability of the matrix to absorb water with respect to the water loss during drying (Prabhanjan et al., 1995) as shown in equation [3.19].

$$\mathrm{WAC} = \frac{m_{rh}\,(100 - M_{in})}{m_{dh}(100 - M_{dh})} \quad [3.19]$$

where m_{rh} is the mass of rehydrated sample (g) M_{in} is the moisture content in wet basis of the sample before drying (g water/g dry matter), m_{dh} is the mass of the dry sample taken for rehydration testing (g) and M_{dh} is the moisture content in wet basis of the dry sample (g water/g dry matter).

Moisture reabsorption ability (RA) was used to determine the ability of the dried product to rehydrate and shows the damage of the tissue caused by drying and rehydration processes (Moreira et al., 2008) as shown in equation [3.20].

$$\mathrm{RA} = (WAC)(DHC) \quad [3.20]$$

Shrinkage, as shown in equation [3.21], was determined by taking the ratio between the sample thickness (*L)* after and before drying (Yan et al., 2008).

$$S = \frac{L_d}{L_o} \quad [3.21]$$

where L_d is the apparent thickness of the EBN sample after drying (m) and L_o is the apparent thickness of the raw EBN sample measured by a vernier calliper (m).

3.5 Biochemical Properties Analyses

3.5.1 Determination of proximate composition

The official methods of the Association of Official Analytical Chemistry (AOAC, 2005) were employed to determine the moisture, protein, fibre, ash content and fat content of the ground EBN dried samples. Moisture content was determined by drying the EBN sample in an oven at 105°C until a constant weight was obtained (Method 934.01). Crude protein content was determined by Kjeldahl's method, using 6.25 as a conversion factor (Method 2001.11). Fibre was determined after digesting a known weight of a fat-free sample in refluxing 1.25% sulphuric acid and 1.25% sodium hydroxide (Method 962.09).

Ash contents were determined by dry ashing in a furnace at 550°C for 18 h (Method 942.05). Crude fat content was calculated from a fraction of lipid extracted from the hydrolysed EBN sample (Wrolstad et al., 2005). The sample was first digested by hydrochloric acid (HCl) before extracting with a mixture of chloroform/methanol (1/1, v/v). Removal of the organic solvent was carried out using a rotary evaporator (Buchi, Switzerland). The analyses were carried out in triplicate and the results were expressed as the percent of dried matter (DM) basis. Finally, the available carbohydrate was obtained by the difference method (subtracting the percent of crude protein, fat, fibre and ash from 100% dry matter) (Wrolstad et al., 2005).

3.5.2 Nitrite analysis

Five grams of EBN dried samples were ground and mixed with 80 ml of ultrapure water in a volumetric flask. Mixture was placed in warm water bath at 80°C for 30-min and shaken once every 5-min to make sure that the solid phase was evenly distributed. The mixture was cooled in room temperature and centrifuged (Eppendorf, Centrifuge 5430, Malaysia) at 10,000 rpm for 15-min. The supernatant fluid was filtered by a membrane filter to get a clear extract and

subjected for ion chromatography (IC) analysis (Dionex IC-90 Ion Chromatograph, Thermo Scientific, US) according to MS 2509:2012(P) (Department of Standards Malaysia, 2012). High capacity anionic exchange column compatible to gradient elution, such as Dionex IonPac As 14a with a conductivity detector was used in anion content analysis. Potassium hydroxide (KOH) elution solution at concentrations of 6 mmol/l and 70 mmol/l was prepared. Its elution gradient was 6 mmol/l for 30-min, 70 mmol/l for 5-min and 6 mmol/l for 5-min, respectively, at flow rate is 1.0ml/min (Department of Standards Malaysia, 2012; Ministry of Health of the People's Republic of China, 2010).

The stock solution of nitrite was diluted with water to prepare a series of standard solutions with nitrite ion concentration of 0 mg/L, 0.02 mg/L, 0.04 mg/L, 0.06 mg/L, 0.08 mg/L, 0.10 mg/L, 0.15 mg/L, 0.20 mg/L, respectively, and with nitrate ion concentration of 0 mg/L, 0.20 mg/L, 0.40 mg/L, 0.60 mg/L, 0.80 mg/L, 1.00 mg/L, 1.50 mg/L, 2.00 mg/L, respectively. The chromatographic diagram of standard solution for each concentration was obtained by the successive injection of samples from the lowest concentration. The calibration curve was plotted using concentration (mg/L) of nitrite ions as abscissa and peak height (μS) and peak area as ordinate for the quantitative of calibration curve (Ministry of Health of the People's Republic of China, 2010).

Fifty microliter of blank solution and 50 μl sample solution were injected into an ion chromatography and the chromatographic diagrams were then recorded. A five points external calibration curves for targeted ions were constructed and used for quantitative determination. The peak height (μS) and peak area were individually measured using the retention time for qualitative analysis. The contents of nitrite (counted on NO_2^- ion) in samples were calculated in accordance with equation [3.22] (Department of Standards Malaysia, 2012; Ministry of Health of the People's Republic of China, 2010).

$$X_{nitrite} = \frac{C\ x\ V\ x\ f\ x\ 1000}{m\ x\ 1000} \quad [3.22]$$

where $X_{nitrite}$ is the content of nitrite or nitrate in sample (ppm), *C* is the content of nitrite or nitrate in samples for measurement (mg/L), *V* is the volume of sample solution (mL), *f* is dilution factor of sample solution, and m is mass of sample taken (g).

The content of NO_2^- in the sample was multiplied by 1.5 to represent the nitrite content (calculation per sodium nitrate). The result was expressed as mean value from three independent determination results under similar conditions (Department of Standards Malaysia, 2012; Ministry of Health of the People's Republic of China, 2010).

3.5.3 Sialic acid analysis

The dried EBN samples were finely ground using a food grinder (Mapporo Star, Blender 800, Malaysia). The finely ground EBN samples were sieved (1mm) and stored inside air tight containers which were kept at ambient temperature until further analysis. The sample treatment and analysis of the raw EBN samples were performed as described by Yu et al. (2000) with some modifications. Ten milligrams of crushed dried raw EBN was weighed and the sample was put into a glass vial with a Teflon sealing screw for derivatization. After 0.5 mL of 1M sulphuric acid-methanol was added, the vial was filled with nitrogen gas and closed tightly. Then the vial was heated in an oven (Memmert, Schwabach, Germany) for 90-min at 90°C. After cooling to room temperature, the solvent in the vial was transferred into a centrifuge tube and 0.3 g of barium carbonate was added to neutralize the sulphuric acid in the solution.

The tube was placed on an ultrasonic vibrator (Interscience, Memmert, Germany) at 100 rpm for 5-min. Sample was then centrifuged at 4,500 rpm for 5-min and

the supernatant was further centrifuged at 14,000 rpm for 6.5-min. The supernatant was then transferred into a test tube with a ground-glass cover. For acetylation, the dried sample was thoroughly shaken and stirred with 1 mL of 1M pyridine and 0.5 mL of 1M acetic anhydride and then incubated at 90°C for 30-min. Sample solution was centrifuged at 14,000 rpm for 10-min during cooling, and the sample was subsequently injected to HPLC for analysis after 1 hour later.

Sialic acid content was analysed using an Agilent 1200 Series HPLC with RI detector for detection. Separation was performed on Agilent Hi-Plex H column (7.7 mm x 300 mm, 8 μm film thickness) with gradient elution of 0.005 M sulphuric acid. The operating conditions were the injection port temperature, 100°C; interface temperature, 70°C; column compartment temperature, 60°C; 0.005 M of sulphuric acid (flow rate of 0.5 ml/min at 60°C and 50 bar; 0.4 µL injection volume). The injector was operated in splitless mode for 1-min after sample injection. Five monoses, namely $_D$-Mannose (Man), D-Galactose (Gal), N-Acetyl-$_D$-galactosamine (AcGal), N-Acetyl-$_D$-glucosamine (AcGlu), and N-Acetyl-neuraminic acid (also called sialic acid, NaNa) derived from the oligosaccharides components of fresh EBN were used as standards. All the standard chemicals were of HPLC grade and was purchased from Sigma (St. Louis, USA).

Table 3.4 Retention time of five monoses as detected by Agilent 1200 Series HPLC with RI detector.

Target Analyte	Retention Time (min)	
	Mean ± SD	RSD (%)
$_D$-Mannose (Man)	8.18 ± 0.2	0.3
D-Galactose (Gal)	8.25 ± 0.1	0.2
N-Acetyl-$_D$-glucosamine (AcGal)	9.94 ± 0.2	0.4
N-Acetyl-$_D$-glucosamine (AcGlu)	10.07 ± 0.3	0.6
N-Acetyl-neuraminic acid (NaNa)	15.01 ± 0.1	0.1

Note: Each value is the mean retention time after three replications.

Identification of compounds was performed by comparing the mass spectra and retention times of the chromatographic peaks with those of standards as shown in Table 3.4. For quantification, the standard method was introduced to compensate the potential sources of errors during the sample detection. Calibration curves of five monoses were obtained from the concentration ratio versus the corresponding peak area ratio of each oligosaccharides to internal standard. Each analysis was carried out in triplicate.

3.5.4 Antioxidant analysis

About 0.5 g of sample powder was weighed in a conical flask and then added with 25 ml of 0.1 M hydrochloric acid (solid-to-solvent ratio of 1:50). The conical flask was sealed with parafilm to avoid any loss of solvent through evaporation (Amarnath, 2004; Wong et al., 2005). Antioxidant in EBN was extracted at 30°C for 45 minutes in the dark, by a hot water bath shaker (Interscience, Memmert, Germany) at 100 rpm (Amarnath, 2004; Wong et al., 2005). The mixture was centrifuged (Eppendorf, Centrifuge 5430, Malaysia) and then the supernatant was filtered by using a membrane filter to get a clear extract. The antioxidant activities were analysed by using three *in-vitro* chemical methods, which are 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay, 1,1-diphenyl-2-pycrylhydrazyl (DPPH) free radical scavenging assay, and Ferric reducing antioxidant power (FRAP) assay.

3.5.4.1 ABTS assay

ABTS radical cation ($ABTS^{+}$) solution was prepared by mixing 7.4 mM $ABTS^{\cdot+}$ solution and 2.6 mM potassium persulfate in equal quantity and the reaction of both solutions was carried out for 12 hour at room temperature in the dark. The reaction product was then diluted by mixing 1 ml $ABTS^{\cdot+}$ solution with 60ml methanol to obtain an absorbance of 1.1 ± 0.02 at 734 nm using spectrophotometer (Hach, DR2800, Germany). Extracts in 150 µl was allowed to

react with 2850 µl of $ABTS^+$ solution for 2 hour in dark condition. Absorbance was taken at 734 nm using spectrophotometer (Hach, DR2800, Germany). A standard curve was constructed by using ABTS stock solution with concentration in the range of 20-100 mg/mL $ABTS^+$ solution as shown in Figure F1 (Appendix F). Results were expressed in mg Trolox Equivalents Antioxidant Capacity (TEAC)/g dry weight.

3.5.4.2 DPPH assay

The DPPH stock solution was prepared by dissolving 24 mg DPPH with 100ml methanol and then stored at -20°C. The working solution was obtained by mixing 10 ml of stock solution with 45 ml methanol to obtain an absorbance of 1.1±0.02 units at 515 nm using a spectrophotometer (Hach, DR2800, Germany). Extracts in 150 µl were allowed to react with 2850 µl of DPPH solution for 24h in the dark. The absorbance was taken at 515 nm using the spectrophotometer. A standard curve was constructed by using DPPH stock solution with concentration in the range of 20-100 mg/mL as shown in Figure F2 (Appendix F). Results were expressed in mg Trolox Equivalents Antioxidant Capacity (TEAC)/g dry weight.

3.5.4.3 FRAP assay

FRAP stock solution was prepared by mixing 300 mM acetate buffer (3.1g sodium acetate trihydrate, $C_2H_3NaO_2.3H_2O$ and 16ml acetic acid, $C_2H_4O_2$) at pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM hydrochloric acid, HCl and 20 mM Iron (III) Chloride Hexahydrate, $FeCl_3.6H_2O$ solution. The working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml Iron (III) Chloride Hexahydrate, $FeCl_3.6H_2O$ solution and then warmed at 37°C before use. Extracts in 150 µl were allowed to react with 2850 µl of the FRAP solution for 30min in the dark condition. The absorbance was taken at 593 nm using a spectrophotometer (Hach, DR2800, Germany). A standard curve was constructed by using FRAP stock solution with concentration in the range of 20-

100mg/mL FRAP solution as shown in Figure F3 (Appendix F). Results were expressed in mg Trolox Equivalents Antioxidant Capacity (TEAC)/g dry weight.

3.5.5 Microbiological analysis

All dried samples were sent to SaniChem Resources Sdn Bhd (Negeri Sembilan, Malaysia) for further analysis on total plate/microbial count, yeast and mould count. For each dried edible bird's nest sample, 10 g was weighed and dispersed aseptically in 90 ml of citrate buffer (2%, w/v) and homogenized in a sterile polyethylene bag using a Stomacher (Seward Laboratory Blender Stomacher 400 Lab Blender UK) for 1.5 min. Serial dilutions were made in 0.1% sterile peptone water and all determinations were made in duplicate. The enumeration of total plate count (Plate Count Agar, Merck, Darmstadt, Germany) at 30°C for 48 hr (Messer et al., 1985), yeast and moulds (Potato Dextrose Agar, Merck, Darmstadt, Germany) at 21°C for 7 days (Frank et al., 1993) were performed.

At the end of the incubation period, colonies were counted and expressed as colony forming units per gram (cfu/g). The mould isolates were purified on potato dextrose agar (PDA) and further sub-cultured on malt extract agar (MEA), Czapek yeast extract (CYA), 25% glycerol nitrate (G25N) and Czapekyeast extract 20% sucrose agar for microscopic examination and identification. The isolated moulds were identified by their morphological characteristics and pigments as described by Samson et al. (2000).

3.6 Storage Stability Test

An ultraviolet C disinfection storage cabinet with an outer dimension (W = 745 mm x H = 725 mm x D = 380 mm) and an inner compartment (W = 690mm x H = 385 mm x D = 290 mm) as shown in Figure 3.3 (and also actual equipment in Appendix B) was fabricated and supplied locally by I-Lab Sdn. Bhd. (Selangor, Malaysia). The equipment was used to store edible bird's nest for the stability

studies. Two ultraviolet C (UVC) lights (23.6 V, 4 W, short-wave UVC radiation with a maximum peak at 253.7 nm) (model TUV 4W FAM, Yanming Lighting, Shanghai, China) of diameter 16 mm are installed on the top of storage cabinet.

The germicidal ultraviolet C (UVC) light system was installed in this storage cabinet to control the microbial activity. In addition, the environment was controlled so that the product can be stored safely in a longer period. A heater was installed in the dryer to further increase the air temperature. The cabinet was designed as a closed loop system whereby the air is circulated within the storage cabinet without any exchange with the surrounding air. The temperature and humidity were controlled using three Proportional Integral Derivative (PID) controllers. The nitrite content of EBN stored in the UV assisted storage cabinet with storage environment control at 27°C and 28.9% RH (method 1) was compared with the samples that were stored by using another two storage methods which are currently practised by the edible bird's nest industry.

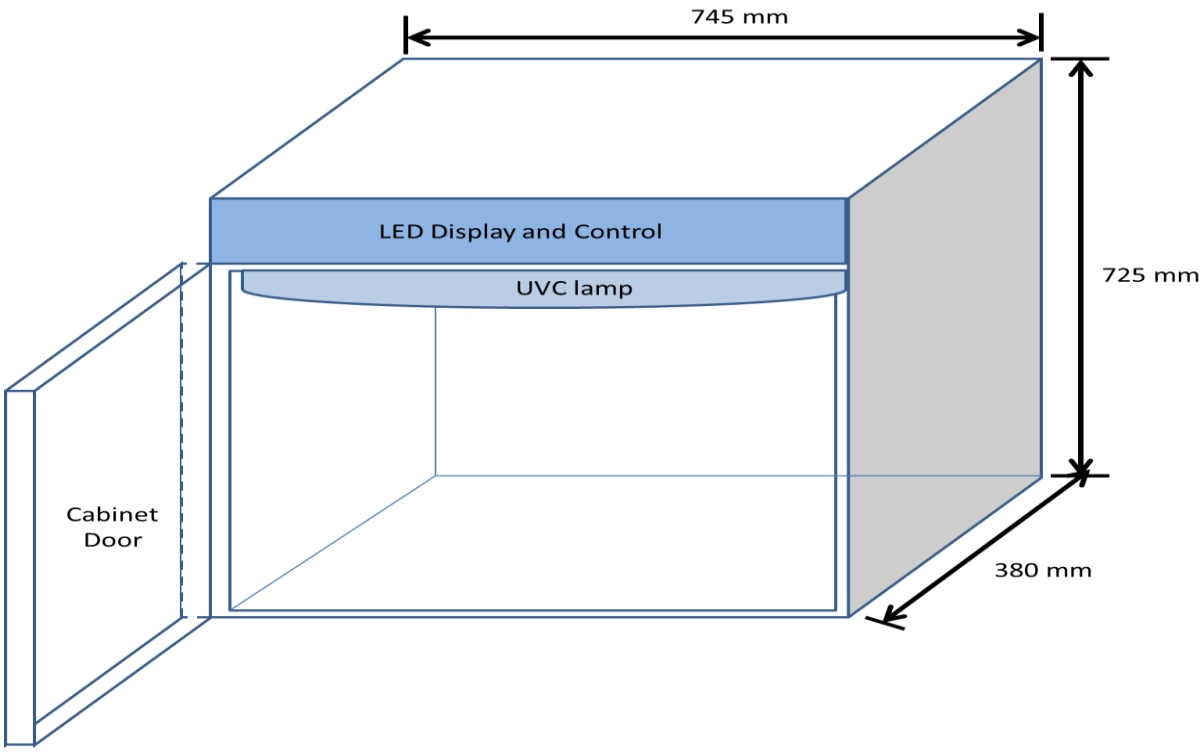


Figure 3.3 Schematic diagram of ultraviolet C disinfection storage cabinet.

The two different storage methods were studied, which were packed in a closed plastic container and stored in a refrigerator at -2°C (method 2) and vacuum sealed plastic packed by vacuum packing machine (800 W, 220 V) (model TVPM-400, Euroasia Food Equipment Sdn. Bhd., Penang, Malaysia) (method 3). The vacuum packing machine has 800 W of heat sealing power and 20 m^3/h of the gas suction speed. At first, the whole piece of EBN sample was covered by two plastic sheets and then placed into the vacuum packaging chamber. The air inside was sucked out by a suction speed of 20 m^3/h at pressure of 1.33kPa and then the two plastic sheets with EBN sample were fully sealed. During storage, the colour analysis was performed in monthly basis. At the end of the storage study, all samples were analysed for final moisture content, water activity, colour changes, nitrite content, yeast and mould contents, sialic acid and antioxidant contents.

3.7 Optimization of IR-UVC Assisted Intermittent Drying

3.7.1 Selection of drying profile and experimental range

The preliminary assessments were carried out to determine several important variables on drying profile and to select suitable experimental range before the study on optimization of intermittent drying. Firstly, drying duration and temperature were determined by comparing drying kinetics of samples in three drying modes, which are fan assisted drying at room temperature (FAN27), IR-UVC assisted drying at 25°C (IR-UVC25) and IR-UVC assisted drying at 40°C (IR-UVC40). After selecting the best drying conditions, the influence of temperature on product quality was studied by considering colour change, sialic acid and antioxidant retention in samples in three continuous drying (FAN27, IR-UVC25 and IR-UVC40). Once the best drying mode and best drying temperature for the intermittent and final drying were determined, the optimization of intermittent drying variables such as intermittent duration, intermittent ratio and intermittent cycle were performed by using response surface methodology (RSM).

3.7.2 Response surface methodology (RSM)

In this study, the drying variables that were considered are intermittent duration (X_1), intermittent ratio (X_2) and intermittent cycle (X_3); while the response variables were total drying time (Y_1), total heating time during intermittent (Y_2), total colour change (Y_3), nitrite content change (Y_4), sialic acid content (Y_5) and antioxidant content (Y_6). A three-level experimental design proposed by Box and Behnken (1960) was applied for response surface fitting, with a design matrix of seventeen trials as shown in Table 3.5 below, including five replicates at central point that were used to estimate experimental error (Montgomery, 2001).

Table 3.5 Experimental design and data for response surface analysis.

Standard	Run	Coded variables			Natural variables		
		X_1	X_2	X_3	X_1	X_2	X_3
14	**1**	0	0	0	390	0.33	2
12	**2**	0	+1	+1	390	0.67	3
2	**3**	+1	-1	0	620	0.20	2
13	**4**	0	0	0	390	0.33	2
17	**5**	0	0	0	390	0.33	2
11	**6**	0	-1	+1	390	0.20	3
10	**7**	0	+1	-1	390	0.67	1
4	**8**	+1	+1	0	620	0.67	2
7	**9**	-1	0	+1	210	0.33	3
8	**10**	+1	0	+1	620	0.33	3
3	**11**	-1	+1	0	210	0.67	2
5	**12**	-1	0	-1	210	0.33	1
15	**13**	0	0	0	390	0.33	2
6	**14**	+1	0	-1	620	0.33	1
16	**15**	0	0	0	390	0.33	2
9	**16**	0	-1	-1	390	0.20	1
1	**17**	-1	-1	0	210	0.20	2

Note: X_1 = Intermittent duration (min); X_2 = Intermittent ratio, α;
X_3 = Intermittent cycle

In order to simplify the calculation, it is appropriate to use coded variables for describing independent variables in the (-1, 1) interval. The independent variables were rescaled therefore 0 is in the middle of the center of the design, and ±1 are the distance from the center with direction. Experimental runs were

randomized, to minimize the effects of unexpected variability in the observed responses. The variables were coded according to the equation [3.23] (Montgomery, 2001).

$$N_{coded} = \frac{(N_i - N_o)}{\Delta N} \quad [3.23]$$

where N_{coded} is the coded value, N_i is the corresponding actual value, N_o is the actual value in the centre of the domain, and ΔN is the increment of X_i corresponding to a variation of 1 unit of x.

The mathematical model corresponding to the Box Behnken design is shown in equation [3.24] (Montgomery, 2001). A generalized quadratic polynomial model equation [3.24] for a three factors response surface analysis was fitted to the experimental data to depict the relationship between the key independent variables and responses (Montgomery, 2001).

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=1+1}^{3} \beta_{ij} X_i X_j + \varepsilon \quad [3.24]$$

where Y is the dependent variables, β_0 is the model constant, β_i, β_{ii} and β_{ij} are the model coefficients which represent linear, quadratic and interaction effects of the variables, and ε is the error.

Analysis of the experimental design data and calculation of predicted responses were carried out using Design Expert software (Version 6.0, Stat-Ease, Inc., Minneapolis, MN). All coefficients in the model were determined from multiple regression analysis and later significances of the coefficients were examined by performing the analysis of variance (ANOVA). Meanwhile, the adequacy of model was determined using sequential sums of squares, lack-of-fit test, coefficient of variation (CV) and correlation coefficient (R^2). Small p-value in sequential sums of squares, insignificant value of lack-of-fit and high value of R^2 (close to unity)

would indicate good fitting of the model to the response. The optimal extraction conditions were then estimated through regression analysis and three-dimensional (3D) response surface plots to visualize the relationship between the response and variable.

Subsequently, an optimization was performed by numerical technique and targeted to obtain the best product quality (minimum colour and nitrite change with maximum retention of sialic acid and antioxidant) at minimum operating cost (minimum total drying time and heating time). The optimum condition was validated by conducting experiments according to the predicted condition that obtained from the response surface methodology.

3.8 Statistical Analyses and Validation of Analytic Methods

The experiments were conducted as a completely randomized design experiment in three replicates. All data were analysed using analysis of variance (ANOVA) in SAS 8.0 statistical data analytical software (SAS Inst., Inc., Cary, N.C., USA) and presented as mean values with standard deviations. Duncan's t-test was used to establish multiple comparisons of mean values at a confidence level of 95% ($p<0.05$). To validate the analytical analyses on the quantification of sialic acid, total antioxidant, nitrite and nitrate contents in edible bird's nest samples, the linearity, sensitivity, precision, repeatability and accuracy of the analytic methods for nitrite and nitrate contents, sialic acid and antioxidant contents were determined (ICH Harmonized Tripartite Guidelines, 2005).

For the linearity, the calibration curves of sialic acid and antioxidant (Appendices D and F) were constructed using a range of concentrations of working standards, and each line was based on six different concentrations. The limit of detection (LOD) and limit of quantification (LOQ) were then used to evaluate the sensitivity (Appendices E and G). Precision studies were carried out to ascertain the

reproducibility of the proposed method. The assay precision was determined by intra-day and inter-day variations, which were performed by analysing standard solutions during a single day (*n=6)* and on three executive days (*n=6)* respectively. For repeatability test, five independent sample solutions were prepared. The accuracy was evaluated as the percentage recovery of analytic methods in the spiked samples. The recoveries were calculated by equation [3.25]. While, relative standard deviation (RSD) was used to describe precision, repeatability and recovery %.

$$Recovery\ \% = \frac{Amount\ Found - Original\ Amount\ in\ Fresh\ Sample}{Amount\ Spiked}\ x\ 100\ \% \qquad [3.25]$$

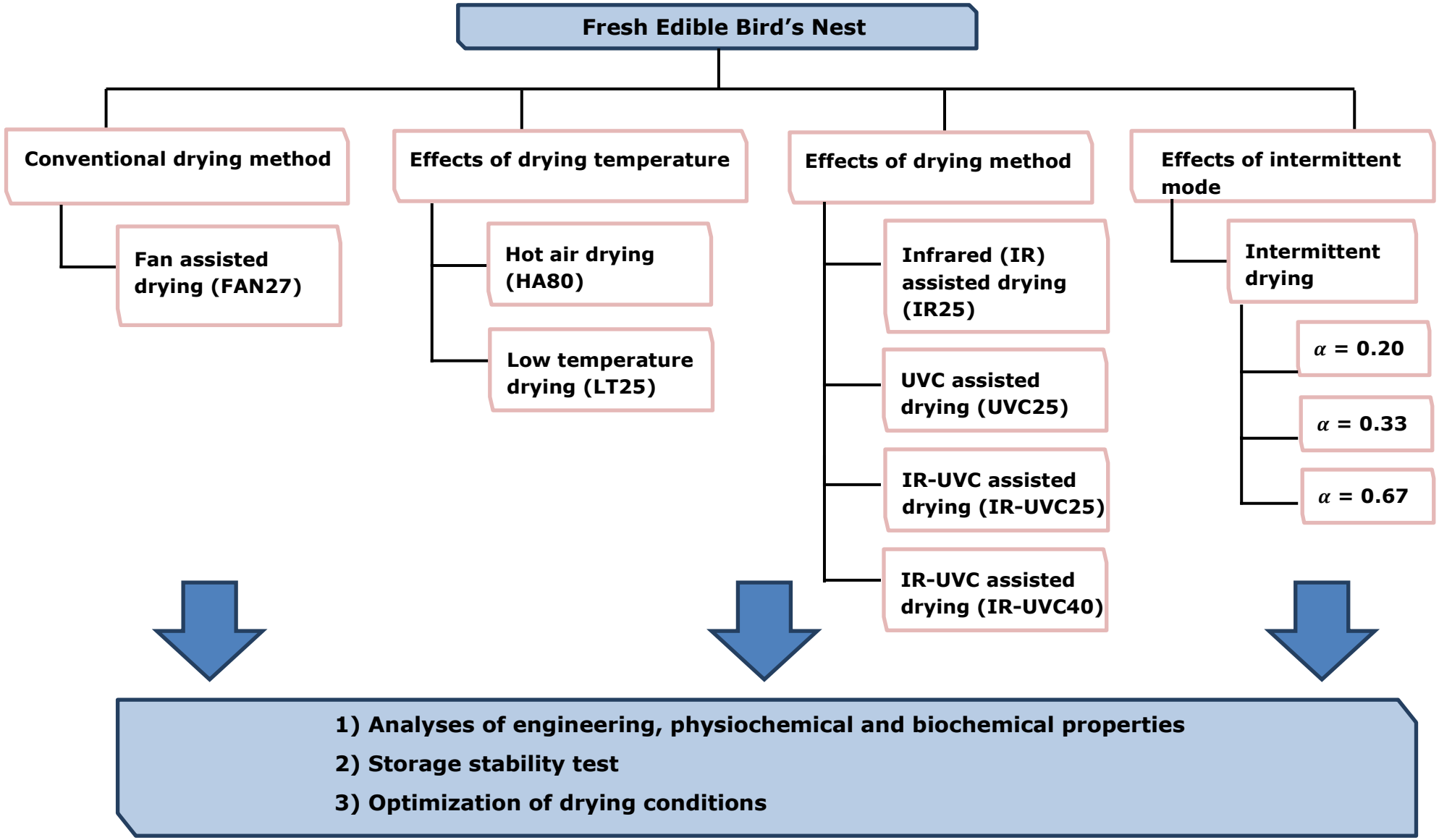


Figure 3.4 Overview of experimental works.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Transport Properties

Processing methods of edible bird's nest (EBN) still remain crude and lack technological advancements. In terms of drying, no studies have attempted to apply advanced drying technology to improve EBN quality (Banyan Bird Nest, 2010; Blissful Bird Nest, 2011; Malaysia Hydroponis, 2013). Drying condition is an important factor in influencing the engineering properties of final product particularly the drying kinetics, effective moisture diffusivity and effective thermal diffusivity. The effects of drying method and drying mode on these product engineering properties are discussed in the following sections.

4.1.1 Drying kinetics and drying rate

Drying medium in IR-UVC assisted drying at low temperature was manipulated to temperatures that were lower than those used in hot air drying in order to study the effects of temperature and relative humidity on drying kinetics and quality of EBN. Drying kinetics of EBN were then compared with hot air drying at 80°C and fan assisted drying at 27°C that are commonly used in food drying (Ong and Law, 2011). Table 4.1 summarizes the total drying time and effective drying time required, as well as equilibrium moisture content (EMC) of dried EBN and the corresponding water activity (a_w) for each drying technique and conditions used.

The variation in moisture content with time for IR-UVC assisted drying of EBN at various levels of intermittency, $\propto$ are expressed in terms of moisture ratio. Total drying time refers to the total process time to reach equilibrium moisture content (EMC) and the effective drying time represents the net drying time of active drying where the external heat source such as infrared (IR) and ultraviolet C (UVC) were applied and drying medium such as low temperature dehumidified air

generated by the IR-UVC assisted dryer system was supplied. Equilibrium moisture content (EMC) of dried EBN sample was targeted as 10%-12%.

Table 4.1 Equilibrium moisture content (EMC), water activity (a_w), total drying time, and effective drying time in accordance with the drying conditions such as drying temperature, relative humidity and intermittency.

Drying Method	T (°C)	RH (%)	∝	EMC (%db)	a_w	Total Drying Time (hr)	Effective Drying Time (hr)
Fan Drying (FAN27)	27	39.7	1.00	11.76	0.57	19.0	19.0
Hot air drying (HA80)	80	8.3	1.00	11.23	0.53	7.5	7.5
Low Temperature Drying (LT25)	25	30.2	1.00	11.36	0.54	15.0	15.0
Infrared Assisted Drying (IR25)	25	29.5	1.00	11.64	0.51	9.5	9.5
UVC Assisted Drying (UVC25)	25	29.3	1.00	10.89	0.46	12.0	12.0
IR-UVC Assisted Drying (IR-UVC40)	40	16.5	1.00 0.67* 0.33* 0.20*	10.93 11.27 10.68 10.04	0.47 0.50 0.51 0.48	9.0 10.5 11.5 13.0	9.0 7.5 5.0 2.6
IR-UVC Assisted Drying (IR-UVC25)	25	27.5	1.00 0.67* 0.33* 0.20*	10.85 11.01 11.19 10.67	0.52 0.53 0.54 0.56	10.5 13.0 15.0 16.5	10.5 8.7 7.4 3.3

*Intermittency, $\alpha = \frac{T_{ON}}{T_{ON} + T_{OFF}}$

where T_{ON} and T_{OFF} are the "on" and "off" periods respectively of the dryer.

4.1.1.1 Effects of drying method and drying temperature

In Figure 4.1, it appears that moisture reduction by hot air drying at 80°C is the fastest, followed by IR-UVC assisted drying at 40°C and 25°C, respectively. Moreover, the drying time was observed to be shorter with IR-UVC assisted drying at 40°C which was 71% as compared to IR assisted and UVC assisted drying. The deviation in moisture reduction rate under the same drying conditions

was confirmed by examining the drying rates of the samples as shown in Figure 4.2. It can be seen that hot air drying at 80°C gave the highest drying rate and then followed by IR-UVC assisted drying at 40°C. The drying rates of the samples dried by UVC assisted drying at 25°C are almost similar to the samples dried by low temperature drying at 25°C.

Hence, it can be seen that drying rates increased with drying temperature. As expected, air temperature is always the key parameter that significantly influences the drying rate. This is because elevated temperature increases the heat transfer to sample, thus resulting in rapid evaporation and high diffusion rate of moisture in the sample and consequently shortening the drying time. However, the temperature must not exceed a certain limit that may deteriorate the final products quality such as the retention of heat-labile antioxidants (Arroqui et al., 2002; Ong and Law, 2011; Ramesh et al., 2001).

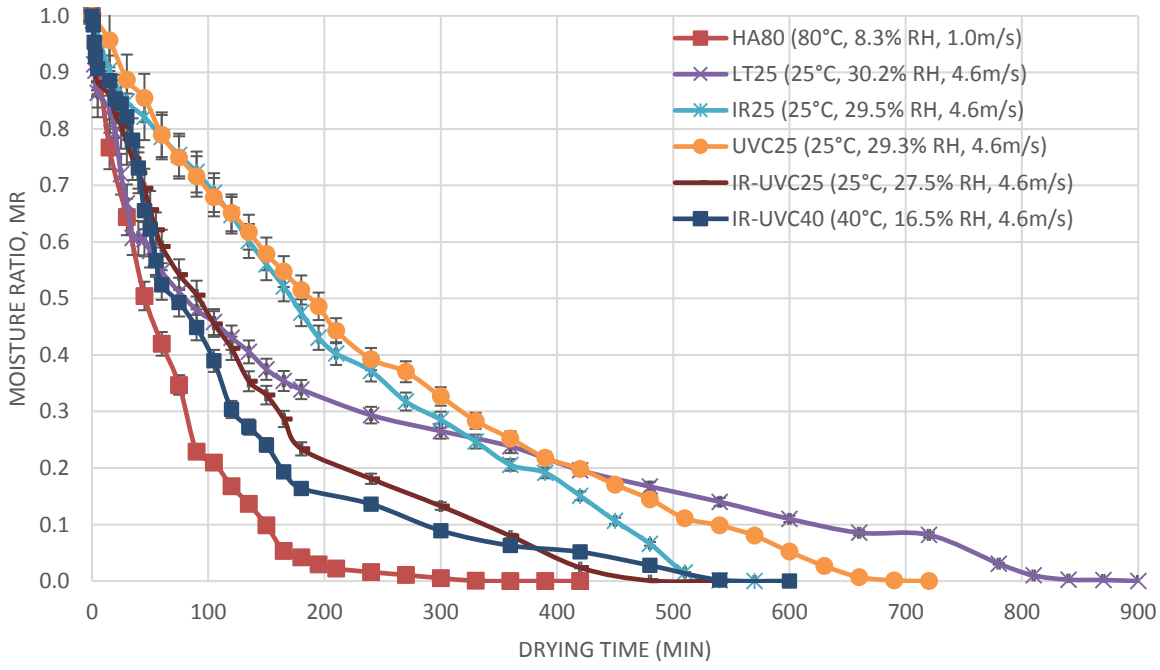


Figure 4.1 Variation of moisture ratios with time in hot air drying and IR-UVC assisted drying at different temperatures.

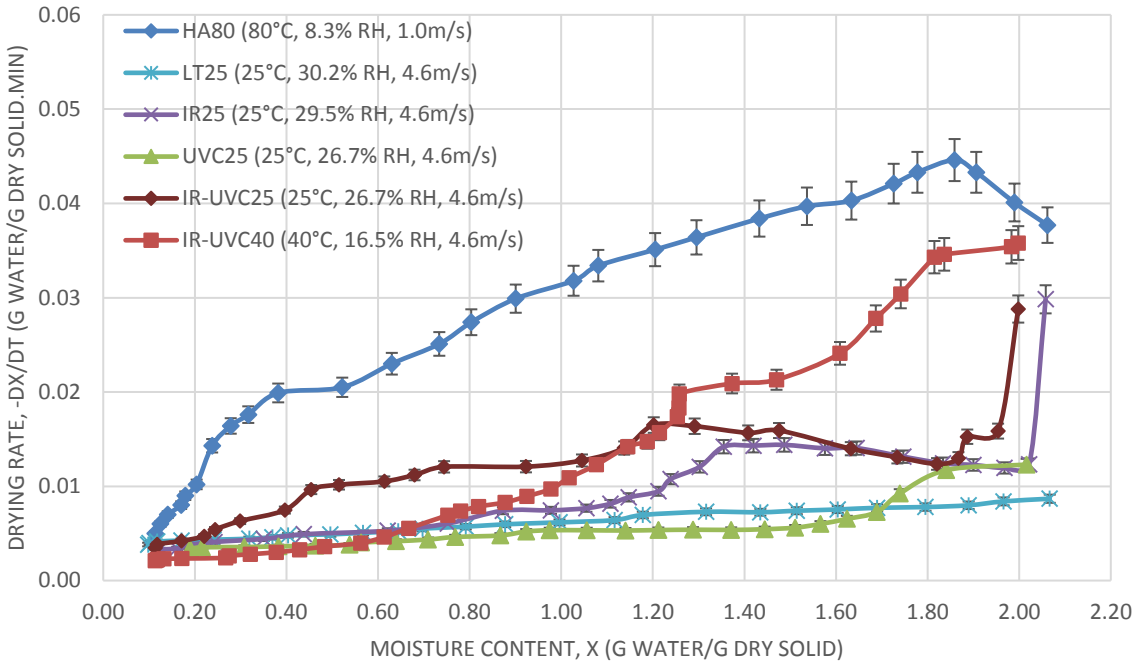


Figure 4.2 Drying rate curves of continuous hot air drying and IR-UVC assisted drying at different temperatures and relative humidity.

It can be seen that hot air drying exhibits convex curves, whereas IR-UVC assisted drying shows concave curves at all drying temperatures. A convex curve indicates a more gradual diminution in drying rate with reduction of moisture content compared to a concave curve. Indirectly, this shows that driving force is higher in the former than in the latter during the internal moisture-controlled drying period (Ong and Law, 2011). Falling rate periods were observed in hot air drying (HA80) and IR-UVC assisted drying at 40°C (IR-UVC40) at moisture content lower than 1.4 g water/g dry solid. However, for low temperature drying at 25°C (LT25) and UVC assisted drying at 25°C (UVC25), constant rate periods were observed at moisture content lower than 1.4 g water/g dry solid. This was due to the low drying temperature (ambient air condition) throughout the drying process which had lesser degree of moisture evaporation. The surface moisture remained saturated for a longer period of time after initial phase of drying and only surface evaporation took place during this period. This conformed to the

recorded mean temperature profile which was almost constant during this period (Figure 4.7).

In addition, it was observed in Figure 4.2 that the drying rate of IR-UVC assisted drying at 40°C was lower than the drying rate of IR-UVC assisted drying at 25°C for moisture content below 1.3 g water/g dry solid. Also, it was observed that drying rate of IR-UVC assisted drying at 25°C was higher than IR or UVC assisted drying at 25°C at the beginning and later stage of drying. The significantly higher drying rate with the assistance of IR and UVC treatment could be attributed to the synergistic effect of IR and air velocity. Rapid diffusion of moisture to the surface of EBN due to the infrared heating and simultaneous removal of moisture from the surface by forced convection resulted in a quicker drying process (Vishwanathan et al., 2010).

In the present study, initial transient periods were observed in hot air drying as shown in Figure 4.2 before it reached the highest drying rate but it was not observed in the IR-UVC assisted drying. This could be due to the higher air velocity employed in the IR-UVC assisted drying compared to hot air drying. Apparently, air velocity plays an important role in influencing the drying rate. A greater driving force for moisture removal on the solid surface could be created by lowering water pressure in the drying air, hence increasing the drying rate (Jangam et al., 2008; Ong and Law, 2011). Drying with high air flow would enhance convective heat transfer to the sample to reach the desired product temperature as well as surface moisture removal in the initial stage of drying. Rapid removal of surface moisture renders the surface in a partially dry condition quickly, in turn, results in the inexistence of constant rate period (Chin et al., 2009; Yaldyz and Ertekyn, 2001).

The main purpose of IR treatment is to speed up drying rate as the radiation energy can be absorbed directly by the drying material without heating up the surrounding air, causing no significant energy loss to the environment (Nattriya and Athapol, 2013). The improvement in the drying rate could be attributed to the altered cell structure such as disruption of membranes of the sample during infrared drying. Hence, the permeability for moisture diffusion across the surface membrane increases and additional paths of moisture diffusion are created as a result of increased volume expansion of the surface membrane after infrared treatment. Upon heating, cells become more rounded (contracted) and cell-to-cell contact decreases. Intercellular spaces at the cell corners increases and thus there is less resistance for moisture diffusion (Goodwin and Jenks, 2005). On the other hand, the main purpose of UVC treatment is to inactivate microorganisms and bacteria e.g. nitro-bacteria, in order to reduce the nitrite content in EBN.

4.1.1.2 Effects of drying mode

Referring to Table 4.1, intermittent IR-UVC assisted drying could shorten the total drying time and also effective drying time required to achieve the desired final moisture content as compared to continuous drying. In all cases as reported for most agricultural products, it can be seen in Figure 4.3 that the moisture ratio of EBN samples decreased exponentially with time (Chin and Law, 2010). Drying kinetics for intermittent IR-UVC assisted drying of EBN at various levels of intermittency are as shown in Figure 4.4. For IR-UVC assisted drying, intermittent drying extends the total drying time but shortens the effective drying time required for the drying process to achieve the desired final moisture content. For instance, the total process time for continuous IR-UVC assisted drying (α = 1.00) at 25°C to reach equilibrium moisture content (10-12% db) decreased 57% when compared with intermittent drying at tempering periods of 4 hours (α = 0.20).

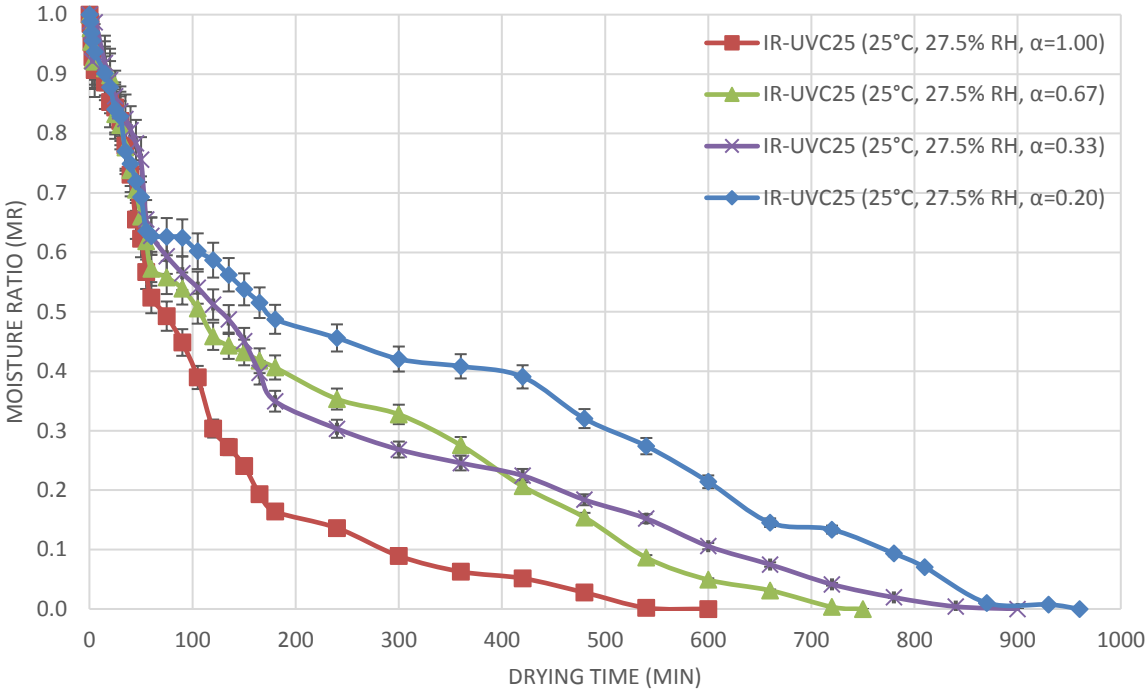


Figure 4.3 Effect of different intermittency on the drying kinetics of intermittent IR-UVC assisted drying of edible bird's nest at 25°C and 27.5% RH.

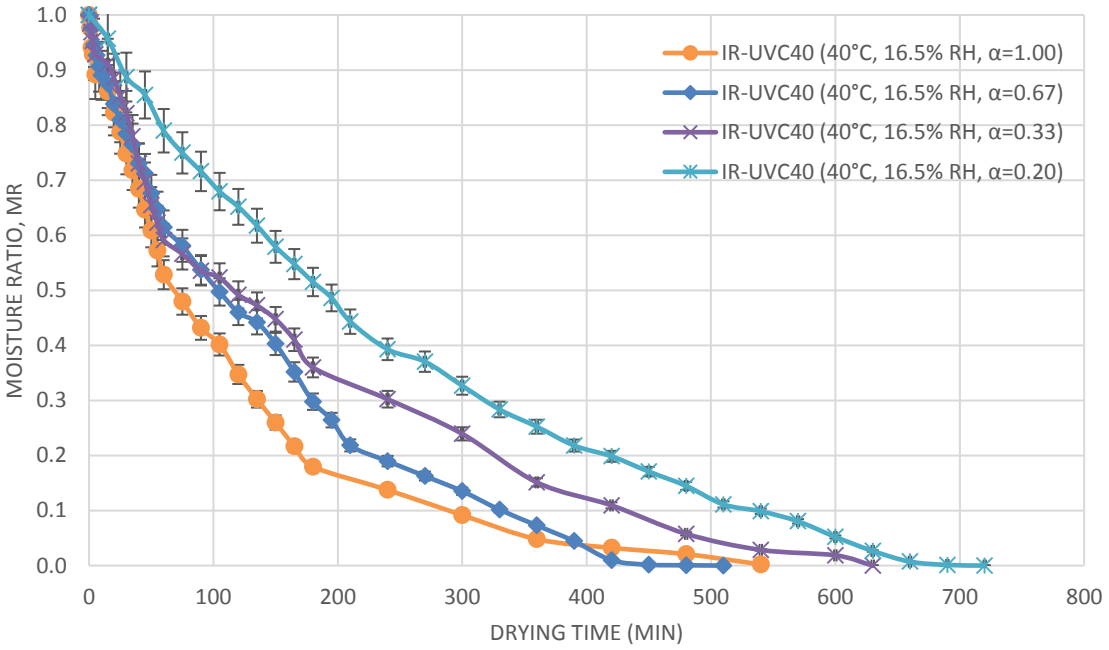


Figure 4.4 Effect of different intermittency on the drying kinetics of intermittent IR-UVC assisted drying of EBN at 40°C and 16.5% RH.

Similarly, intermittent drying at 40°C prolonged the total drying time by 44%, 28% and 17% for α = 0.2, α = 0.33 and α = 0.67, respectively. However, the effective drying time was observed to increase from 17% to 69% and 22.2% to 71% for both intermittent drying at 25°C and 40°C, respectively, when the tempering periods were increased from 1 hr (α = 0.67) to 4 hr (α = 0.2). It appears that commencing a drying process at low temperature, near ambient temperature in the present study, did not favour the removal of surface water although the drying process was conducted in a convective environment.

Nevertheless, the total drying time of intermittent mode IR-UVC assisted drying was observed to be shorter compared to continuous drying. It is interesting to observe that the absence of heat flux in the forced air supply during tempering period not only shorten the effective drying time but also provide sufficient resting for the food sample. The result indicates that heating can be interrupted temporarily at some stages of drying to allow the moisture to transfer from the inner core of the food matrix to the exposed surface (Ong and Law, 2011). Intermittent supply of heat flux during drying period where moisture transport is solely controlled by internal moisture diffusion is advantageous in avoiding overheating of material surface and also minimizing product shrinkage.

Figures 4.5 and 4.6 show the variation of drying rates with moisture content in intermittent IR-UVC assisted drying at 40°C and 25°C. It can be seen that drying rate kicked off quite high in the early stage of drying when the moisture content was still high. However, it is also observed that drying rate decreased significantly when the forced air flow and infrared were halted in the tempering period and increased when the forced air flow and infrared resumed. A similar fluctuation in drying rates is observed in the subsequent cycles but at a much lower magnitude.

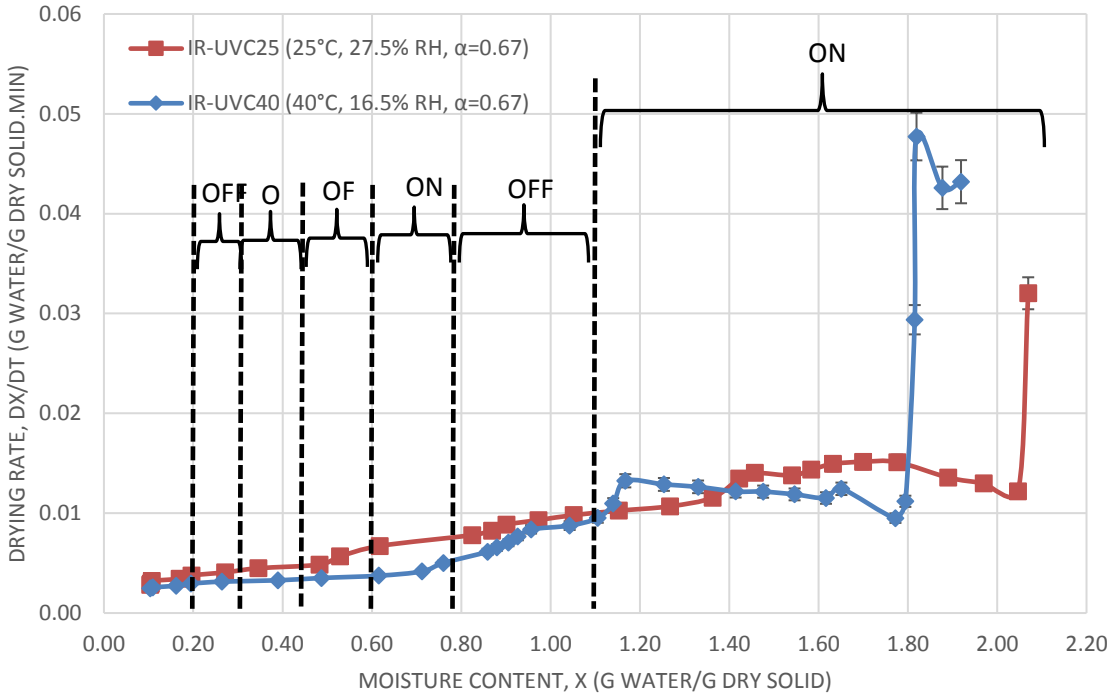


Figure 4.5 Drying rate curves of intermittent IR-UVC assisted drying at 25°C and 40°C, respectively at intermittency of 0.67 (tempering period of 1 hr after every 2 hrs).

This could be due to in each tempering period, internal moisture was given more time to diffuse from the core to the surface of the edible bird's nest sample, which in turn increased the surface moisture content and consequently enhanced drying rate and shortened the drying time in the subsequent active drying period (Chin and Law, 2010). Furthermore, at the early stage of tempering period, noticeable but low removal rate of moisture was observed in Figures 4.5 and 4.6. However, towards the latter stage of tempering, removal of moisture became negligible but internal moisture still slowly migrated toward the surface.

Nonetheless, the variation of drying rates during periodic air supply was not significantly influenced by the movement of convective air beyond the moisture content of 0.60 g water/g dry solid. The result suggests that during low temperature drying (i.e. 25°C), forced air is important in the initial stage of

drying to speed up the drying process and intermittent forced air supply can be beneficial in the latter stage of drying.

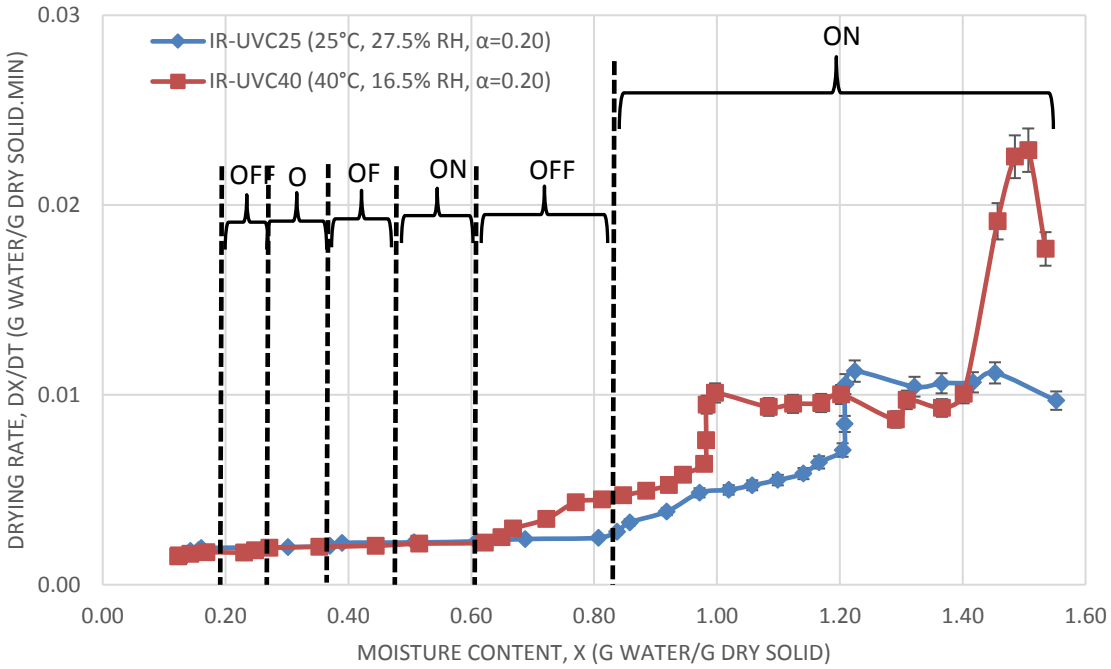


Figure 4.6 Drying rate curves of intermittent IR-UVC assisted drying at 25°C and 40°C, respectively at intermittency of 0.20 (tempering period of 4 hrs).

Forced air can be halted during the diffusion of internal moisture to the material surface and is resumed after the tempering period in order to remove the surface vapour that is accumulated during the tempering period. Rapid drying with low relative humidity coupled with forced air ventilation is preferred in the initial stage of drying because this speeds up the process of surface water removal. In the intermediate stage, where internal moisture movement dominates the drying rate, it is suggested that intermittent drying mode should be applied to provide a tempering period for moisture to diffuse to the surface. Energy saving is possible at this stage by cutting off the heating and air movement intermittently. As for the final stage of drying, the effect of temperature prevails when the moisture content is low and high temperature is favourable in removing the residual moisture.

4.1.2 Effective moisture diffusivity

Table 4.2 shows the effective moisture diffusivity values estimated at the respective product temperatures. The values obtained in this study ranged from 1.0 x 10^{-11} m^2s^{-1} to 1.5 x 10^{-9} m^2s^{-1} and these values are within those reported for most biological materials (Zogzas et al., 1996). It was reported that moisture diffusivities in food materials range from 10^{-13} to 10^{-6} m^2s^{-1} and that almost 82% are within the region 10^{-11} to 10^{-8} m^2s^{-1} (Marinos-Kouris and Maroulis, 2007). The dependency on temperature is also clearly observed as the effective diffusivity values increased with temperature. By plotting In $Deff$ versus $\frac{1}{T}$, a linear relationship was obtained with high value of coefficient of determination (R^2 > 0.96). Activation energy (E_a) was estimated ranging from 30 kJ/mol to 34 kJ/mol, which is within the range reported for most biological materials from 12 kJ/mol to 51 kJ/mol (Senadeera et al., 2003); while the diffusivity constant (D_o) was estimated ranging from 6.7 x 10^{-6} m^2s^{-1} to 3.4 x 10^{-5} m^2s^{-1}.

Table 4.2 Effective moisture diffusivity values estimated for various drying experiment and respective coefficient of determination, R^2.

Drying method	α	Effective drying/ tempering period	Effective moisture diffusivity, D_{eff} (m^2/s)	R^2
Fan Drying at 27°C (FAN27)	1.00	19 hr (drying)	2.94 x 10^{-10}	0.9987
Hot air drying at 80°C (HA80)	1.00	7.5 hr (drying)	1.50 x 10^{-9}	0.9964
Infrared Assisted Drying at 25°C (IR25)	1.00	9.5 hr (drying)	2.31 x 10^{-10}	0.9976
UVC Assisted Drying at 25°C (UVC25)	1.00	12 hr (drying)	2.35 x 10^{-10}	0.9981
IR-UVC Assisted Drying at 40°C (IR-UVC40)	1.00	9 hr (drying)	4.58 x 10^{-10}	0.9996
	0.67	2 hr (drying)	4.58 x 10^{-10}	0.9999
		1 hr (tempering)	2.91 x 10^{-10}	0.9985
		2 hr (drying)	4.34 x 10^{-10}	0.9999
		1 hr (tempering)	2.56 x 10^{-10}	0.9991

		2 hr (drying)	4.42×10^{-10}	0.9998
		1 hr (tempering)	2.30×10^{-10}	0.9996
		1.5 hr (drying)	4.61×10^{-10}	0.9989
	0.33	1 hr (drying)	4.42×10^{-10}	0.9999
		2 hr (tempering)	2.20×10^{-10}	0.9991
		1 hr (drying)	4.32×10^{-10}	0.9986
		2 hr (tempering)	1.99×10^{-10}	0.9974
		1 hr (drying)	4.20×10^{-10}	0.9974
		2 hr (tempering)	1.96×10^{-10}	0.9925
		1 hr (drying)	4.42×10^{-10}	0.9999
		1.5 hr (tempering)	2.32×10^{-10}	0.9997
	0.20	1 hr (drying)	4.35×10^{-10}	0.9980
		4 hr (tempering)	0.98×10^{-10}	0.9990
		1 hr (drying)	4.42×10^{-10}	0.9986
		4 hr (tempering)	0.84×10^{-10}	0.9992
		1 hr (drying)	4.32×10^{-10}	0.9993
		2 hr (tempering)	1.10×10^{-10}	0.9994
IR-UVC Assisted Drying at 25°C (IR-UVC25)	1.00	10.5 hr (drying)	3.24×10^{-10}	0.9993
	0.67	2 hr (drying)	3.10×10^{-10}	0.9999
		1 hr (tempering)	2.59×10^{-10}	0.9998
		2 hr (drying)	3.28×10^{-10}	0.9997
		1 hr (tempering)	2.96×10^{-10}	0.9996
		2 hr (drying)	3.26×10^{-10}	0.9994
		1 hr (tempering)	2.63×10^{-10}	0.9993
		2 hr (drying)	3.09×10^{-10}	0.9992
		1 hr (tempering)	2.48×10^{-10}	0.9993
		1 hr (drying)	3.26×10^{-10}	0.9997
	0.33	1 hr (drying)	3.57×10^{-10}	0.9999
		2 hr (tempering)	1.25×10^{-10}	0.9999
		1 hr (drying)	3.41×10^{-10}	0.9998
		2 hr (tempering)	1.35×10^{-10}	0.9998
		1 hr (drying)	3.49×10^{-10}	0.9999
		2 hr (tempering)	1.43×10^{-10}	0.9999
		1 hr (drying)	3.57×10^{-10}	0.9997
		2 hr (tempering)	1.31×10^{-10}	0.9996
		1 hr (drying)	3.37×10^{-10}	0.9993
		2 hr (tempering)	1.23×10^{-10}	0.9991
	0.20	1 hr (drying)	3.09×10^{-10}	0.9999
		4 hr (tempering)	0.53×10^{-10}	0.9999
		1 hr (drying)	3.13×10^{-10}	0.9986
		4 hr (tempering)	0.68×10^{-10}	0.9991
		1 hr (drying)	3.10×10^{-10}	0.9982
		4 hr (tempering)	0.49×10^{-10}	0.9990
		1 hr (drying)	3.28×10^{-10}	0.9989

As can be seen from Table 4.2, D_{eff} values increased with drying temperature in both continuous IR-UVC assisted drying at 40°C and hot air drying at 80°C with

values ranging from 4.58 x 10^{-10} m^2s^{-1} to 1.50 x 10^{-9} m^2s^{-1}. This result is in agreement with the results reported in other studies, where the drying temperature is a key accelerator of moisture diffusion in food matrix at which the rate of moisture migration would significantly increase at higher drying temperature (Aghbashlo et al., 2008; Marinos-Kouris and Maroulis, 2006). During intermittent IR-UVC assisted drying, D_{eff} values was slightly higher than that during tempering in stagnant air. This is due to the high airflow and input of heat flux during forced air drying periods that increases the evaporation rate at the sample surface and hence promotes internal moisture diffusion to the surface.

On the other hand, extended tempering periods at $\alpha = 0.33$ and $\alpha = 0.20$ without heat flux input and airflow supply could significantly reduce mass transfer and moisture saturation at the sample surface, hence suppressed moisture diffusion from the core to sample surface due to lower driving force. Besides, D_{eff} values that were observed in the first two hours of intermittent drying could be an indicative of self-moisture diffusion without external energy input which could be due to the high moisture gradient of sample in the initial stage of drying (Marinos-Kouris and Maroulis, 2006).

Furthermore, D_{eff} values of samples by IR-UVC assisted drying at 25°C were higher than the samples dried by fan assisted drying at 27°C. Despite lower drying temperature in IR-UVC assisted drying, higher moisture diffusivity was obtained. This could be due to the radiation energy of infrared absorbed directly by the drying material without heating the surrounding air, causing no significant energy loss to environment. Hence, the permeability for moisture diffusion across surface membrane increases and additional diffusion paths are created due to the increased volume expansion of the surface membrane after infrared treatment (Nattriya and Athapol, 2013).

4.1.3 Effective thermal diffusivity

Table 4.3 shows the effective thermal diffusivity values obtained at the respective drying air temperature and product temperatures. The values obtained in this study from various drying process range from 6.74 x 10^{-11} m^2/s to 6.50 x 10^{-10} m^2/s are relatively similar to the effective thermal diffusivity values of most agricultural products (1.5 x 10^{-10} m^2/s to 1.7 x 10^{-9} m^2/s) (Wong et al., 2001).

Table 4.3 Effective thermal diffusivity values estimated for various drying experiment and respective coefficient of determination, R^2.

Drying method	α	Effective drying/ tempering period	Effective thermal diffusivity, α_{eff} (m^2/s)
Fan Drying at 27°C (FAN27)	1.00	19 hr (drying)	6.74 x 10^{-11}
Hot air drying at 80°C (HA80)	1.00	7.5 hr (drying)	6.50 x 10^{-10}
Infrared Assisted Drying at 25°C (IR25)	1.00	9.5 hr (drying)	3.45 x 10^{-10}
UVC Assisted Drying at 25°C (UVC25)	1.00	12 hr (drying)	2.94 x 10^{-10}
IR-UVC Assisted Drying at 40°C (IR-UVC40)	1.00	9 hr (drying)	5.00 x 10^{-10}
	0.67	2 hr (drying)	4.51 x 10^{-10}
		1 hr (tempering)	2.20 x 10^{-10}
		2 hr (drying)	4.65 x 10^{-10}
		1 hr (tempering)	2.41 x 10^{-10}
		2 hr (drying)	4.20 x 10^{-10}
		1 hr (tempering)	2.04 x 10^{-10}
		1.5 hr (drying)	4.15 x 10^{-10}
	0.33	1 hr (drying)	4.44 x 10^{-10}
		2 hr (tempering)	2.10 x 10^{-10}
		1 hr (drying)	4.41 x 10^{-10}
		2 hr (tempering)	2.08 x 10^{-10}
		1 hr (drying)	4.39 x 10^{-10}
		2 hr (tempering)	2.00 x 10^{-10}
		1 hr (drying)	4.36 x 10^{-10}
		1.5 hr (tempering)	2.00 x 10^{-10}
	0.20	1 hr (drying)	4.34 x 10^{-10}
		4 hr (tempering)	0.97 x 10^{-10}
		1 hr (drying)	4.34 x 10^{-10}
		4 hr (tempering)	1.00 x 10^{-10}
		1 hr (drying)	4.40 x 10^{-10}
		2 hr (tempering)	1.02 x 10^{-10}

IR-UVC Assisted Drying at 25°C (IR-UVC25)	1.00	10.5 hr (drying)	3.65×10^{-10}
	0.67	2 hr (drying)	3.59×10^{-10}
		1 hr (tempering)	2.78×10^{-10}
		2 hr (drying)	3.58×10^{-10}
		1 hr (tempering)	2.70×10^{-10}
		2 hr (drying)	3.60×10^{-10}
		1 hr (tempering)	2.68×10^{-10}
		2 hr (drying)	3.54×10^{-10}
		1 hr (tempering)	2.60×10^{-10}
		1 hr (drying)	3.50×10^{-10}
	0.33	1 hr (drying)	3.14×10^{-10}
		2 hr (tempering)	1.35×10^{-10}
		1 hr (drying)	3.15×10^{-10}
		2 hr (tempering)	1.30×10^{-10}
		1 hr (drying)	3.19×10^{-10}
		2 hr (tempering)	1.41×10^{-10}
		1 hr (drying)	3.23×10^{-10}
		2 hr (tempering)	1.41×10^{-10}
		1 hr (drying)	3.20×10^{-10}
		2 hr (tempering)	1.39×10^{-10}
	0.20	1 hr (drying)	2.99×10^{-10}
		4 hr (tempering)	0.78×10^{-10}
		1 hr (drying)	3.01×10^{-10}
		4 hr (tempering)	0.85×10^{-10}
		1 hr (drying)	3.10×10^{-10}

The results indicate that effective thermal diffusivity would increase with increasing temperature. This can be seen clearly from the hot air dried samples at 80°C and IR-UVC assisted dried samples at 40°C, where thermal diffusivity increases from 5.00×10^{-10} m^2/s to 6.50×10^{-10} m^2/s when the drying temperature increases from 40°C to 80°C. As reported from various studies, drying temperature is the key factor to accelerate the thermal diffusion in food matrix (Wang et al., 2001; Yibas et al., 2009). The plots of temperature evolution for samples under various drying experiments are as shown in Figures 4.7-4.9.

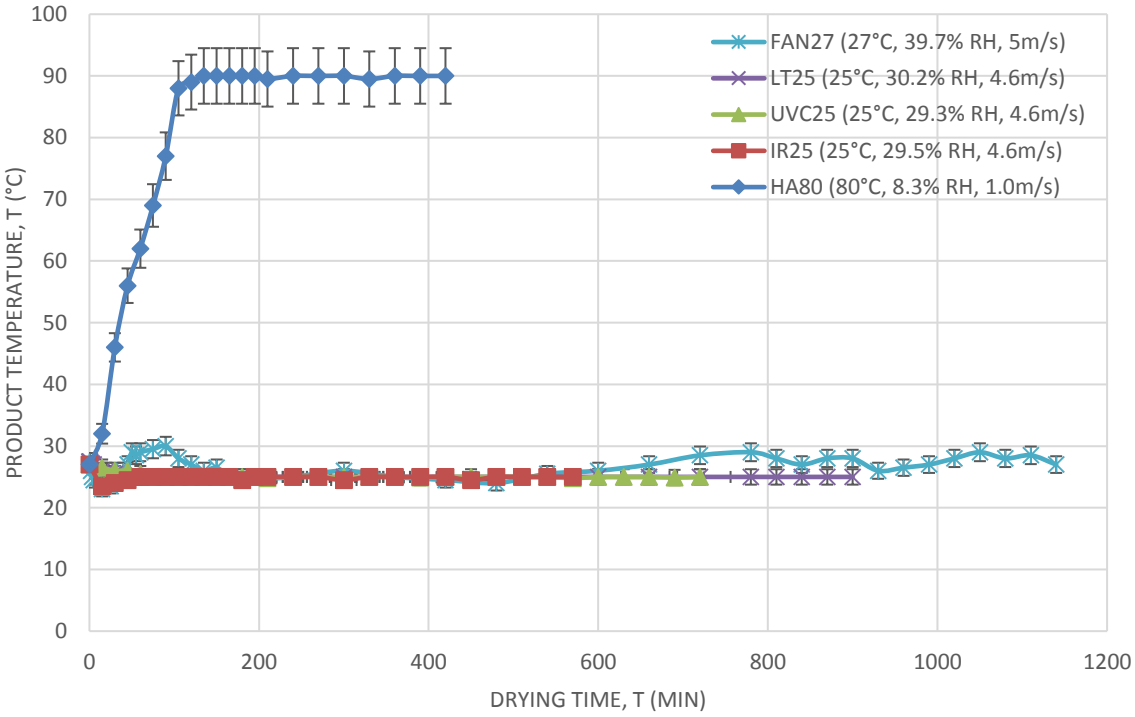


Figure 4.7 Temperature profiles of samples in continuous fan assisted (FAN27), IR assisted (IR25), UVC assisted (UVC25) and hot air drying (HA80) at various drying temperatures.

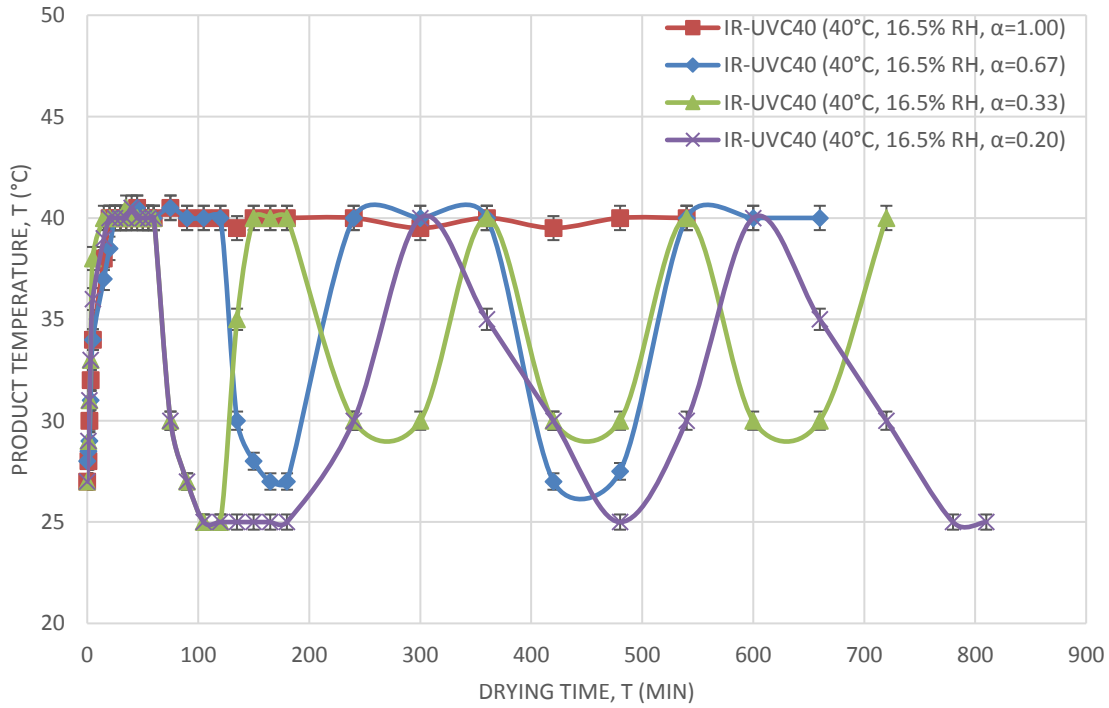


Figure 4.8 Temperature profiles of samples in intermittent drying mode of IR-UVC assisted drying at 40°C, 16.5% RH and α = 0.20 – 1.00.

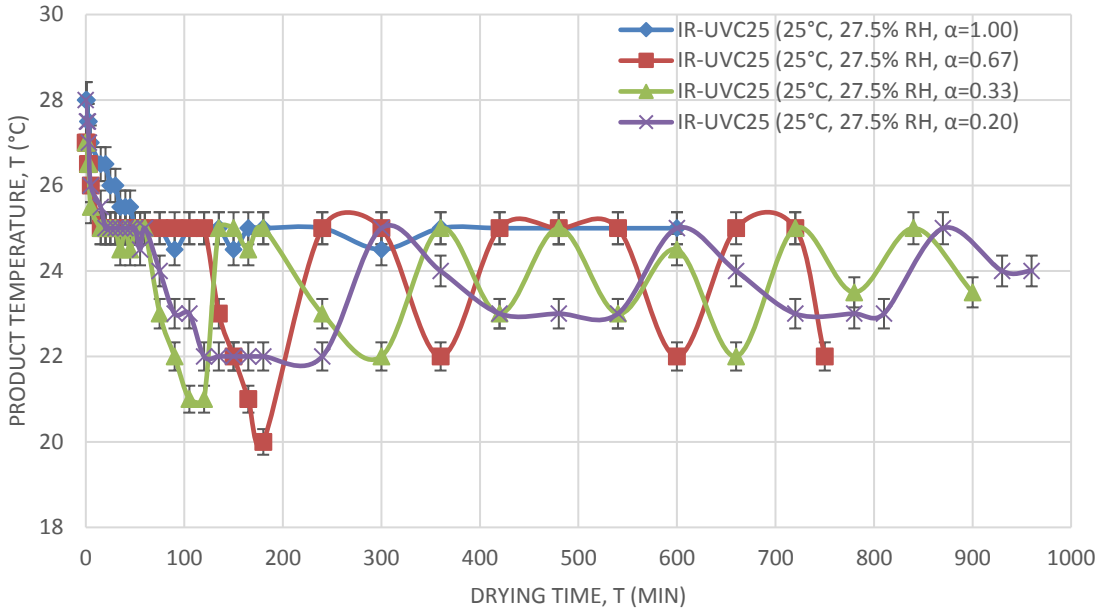


Figure 4.9 Temperature profiles of samples in intermittent drying mode of IR-UVC assisted drying at 25°C, 27.5% RH and α = 0.20 – 1.00.

As shown in Figure 4.7, sample temperature in continuous fan assisted drying (FAN27), UVC assisted drying (UVC25) and hot air drying (HA80) increased very slowly at the beginning of drying due to thermal lag from sample surface to the centre vicinity. Longer time is therefore taken for the heat flux from surrounding drying air to conduct into the centre of the sample. As drying progressed, the sample temperature in continuous drying mode in Figure 4.7 would stabilize at a value of the drying air temperature and eventually approach the temperature of surrounding drying air towards the end of drying. On the other hand, sample temperature in both continuous and intermittent modes of IR-UVC assisted drying at 40°C increased gradually at the beginning of drying (Figure 4.8). Since there is rapid uniform heating and IR radiation that penetrates directly into the inner layer of the sample without heating the surrounding air, hence, uniform temperature distribution can be achieved by IR drying as compared to other drying techniques (Riadh et al., 2015; Sakai and Hanzawa, 1994).

As shown in Figures 4.8 and 4.9, sample temperature as well as the effective thermal diffusivities (α_{eff}) in intermittent IR-UVC assisted drying at 25°C and

40°C fluctuated with drying temperature. The fluctuation of α_{eff} during effective heating time and tempering indicates that there was disrupted heating of sample during the intermittent modes. In turn, less thermal damages to the samples as the total drying time and the average heating flux were reduced and hence heat sensitive bioactive ingredients in EBN such as sialic acid and antioxidant compounds could be retained.

4.2 Physiochemical Properties

4.2.1 Colour alteration

The colour of fresh EBN is creamy-beige, with average *L*, a*, b** values of 58.7 ± 2.62, 0.80 ± 0.21, 12.1 ± 2.85, respectively. The colour change of EBN in this study was compared with the colour of dried samples obtained from continuous hot air drying at 80°C and fan assisted drying. The colour *(CIE)* parameters *L*, a* and b** values and other derived parameters such as total colour change *(ΔE)* and chroma for intermittent IR-UVC assisted drying at different temperature, relative humidity and intermittencies were calculated using equations [3.15] and [3.16].

Generally, browning effect was observed in all samples after drying process; however, it varies among the dried samples depending on drying temperature and drying method. Fan-assisted dried samples (FAN27) show the most yellowish colour as compared to dried samples from other drying methods and then followed by hot air dried samples (HA80). It can be seen from Table 4.4 that *ΔE** values of fan-assisted dried samples increased significantly *($p<0.05$)* with the longest drying period. This indicates that fan-assisted dried samples could have suffered from non-enzymatic browning due to the oxidation of tyrosine without the presence of polyphenoloxidase enzyme (PPO), followed by polymerization during the long hour drying process. Hence, the formation of browning products (melanin) caused EBN discolouration. During fan assisted drying, the presence of

oxygen from air speeds up the first steps in the biochemical conversion of phenolics in EBN sample, such as tyrosine to produce quinones, which undergo further polymerization to yield dark, insoluble polymers (melanins) (Martin et al., 2001; Sugumaran, 2002).

Table 4.5 shows that the colour changes are mainly contributed by the L^* and b^* colour parameters, which increased significantly $(p<0.05)$ in sample HA80, revealing that the severe colour change due to thermal effect (Maskan, 2001). However, no consistent change could be observed in b^* values of other samples. This suggests that intermittent IR-UVC assisted drying could preserve product colour better by avoiding Maillard browning reaction which often occurs in high temperature drying (Chua et al., 2000b; 2001a). Processing parameters affecting Maillard reaction are primarily drying temperature and the duration of the heat treatment (Leber et al., 1999). Based on Table 4.4, it can be seen that ΔE values of dried samples from intermittent IR-UVC drying (IR-UVC25 and IR-UVC40) are significantly $(p<0.05)$ lower than that in continuous mode. This could be due to the shorter heating period on samples by cutting off the heat flux intermittently and hence minimize the browning effect.

Intermittent drying can decrease product surface temperature and increase the rate of moisture removal. Intermittent mode could result in reduction of overall colour change up to 80% as compared to continuous IR-UVC assisted drying and hot air drying. Reduction of total colour change is more prominent for intermittent low temperature dried EBN at 40°C and 25°C, which is up to 83.8% at α = 0.20. This indicates that a long tempering period with a short effective drying time can effectively retard non-enzymatic browning reactions by reducing the total sensible heat transferred from the drying air to the product during drying. These results are consistent with the findings reported by Jangam et al. (2008), Perera and Rahman (1997) and Phoungchandang et al. (2009).

Table 4.4 Colour parameters of dried edible bird's nest that dried by intermittent IR-UVC assisted drying compared to continuous IR-UVC assisted drying and hot air drying.

Drying Methods	Colour Parameters			Total Colour Change, ΔE	Chroma	Hue Angle
	L^*	a^*	b^*			
Fresh Edible Bird's Nest	58.7 ± 2.62^{b}	0.80 ± 0.21^{a}	12.1 ± 2.85^{b}	0.00 ± 1.23^{a}	12.08 ± 2.21^{de}	86.20°± 0.11^{ab}
Dried Edible Bird's Nest Fan drying at 27°C and 39.7% RH	81.6 ± 3.71^{a}	0.80 ± 0.70^{a}	33.0 ± 3.21^{d}	31.04 ± 2.24^{b}	33.00 ± 1.80^{c}	88.61°± 1.51^{c}
Hot air drying (Continuous) at 80°C and 8.3% RH	75.9 ± 2.59^{b}	0.70 ± 0.28^{a}	25.7 ± 9.56^{bc}	21.96 ± 1.47^{c}	25.71 ± 4.75^{bd}	88.44°± 1.52^{ab}
Infrared (IR) assisted drying at 25°C and 29.5% RH						
α = 1.00 (Continuous)	82.4 ± 0.45^{ab}	0.70 ± 0.69^{a}	36.2 ± 2.06^{bc}	33.84 ± 3.38^{b}	36.21 ± 1.07^{c}	88.89°± 1.23^{c}
UVC assisted drying at 25°C and 29.3% RH						
α = 1.00 (Continuous)	82.1 ± 0.99^{a}	0.80 ± 0.52^{a}	35.7 ± 4.27^{bc}	33.27 ± 1.00^{b}	35.71 ± 2.69^{c}	88.72°± 0.95^{c}
IR-UVC assisted drying at 40°C and 16.5% RH						
α = 0.20	61.3 ± 6.61^{b}	0.80 ± 0.17^{a}	16.4 ± 6.52^{b}	5.07 ± 4.10^{a}	16.42 ± 3.48^{bd}	87.38°± 2.07^{ab}
α = 0.33	61.7 ± 5.86^{c}	0.70 ± 0.21^{a}	18.0 ± 6.54^{bc}	6.66 ± 3.57^{a}	18.01 ± 2.50^{de}	87.77°± 1.98^{ab}
α = 0.67	60.5 ± 2.47^{c}	0.80 ± 0.07^{a}	17.5 ± 7.81^{bc}	5.74 ± 5.20^{a}	17.52 ± 6.41^{bd}	87.21°± 0.76^{a}
α = 1.00 (Continuous)	67.5 ± 2.42^{bc}	0.70 ± 0.26^{a}	20.8 ± 2.55^{bc}	26.55 ± 5.46^{c}	30.81 ± 0.12^{de}	88.70°± 0.54^{b}
IR-UVC assisted drying at 25°C and 27.5% RH						
α = 0.20	58.9 ± 2.75^{b}	0.80 ± 0.56^{a}	17.6 ± 0.84^{bc}	5.55 ± 1.08^{a}	17.62 ± 2.88^{bcd}	87.40°± 0.79^{ab}
α = 0.33	62.3 ± 3.69^{b}	0.70 ± 0.39^{a}	18.9 ± 4.11^{bc}	7.74 ± 0.69^{a}	18.91 ± 3.66^{bcd}	87.88°± 1.25^{ab}
α = 0.67	65.8 ± 4.57^{b}	0.80 ± 0.16^{a}	21.4 ± 2.96^{b}	11.74 ± 6.53^{a}	21.41 ± 7.49^{ce}	87.86°± 2.13^{ab}
α = 1.00 (Continuous)	60.4 ± 0.71^{ab}	0.80 ± 0.10^{a}	22.7 ± 3.21^{bc}	29.96 ± 1.76^{c}	32.71 ± 3.30^{de}	88.60°± 1.54^{ab}

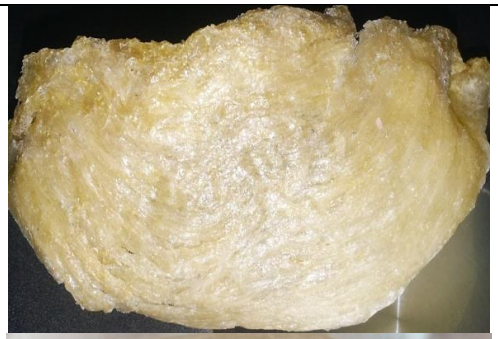
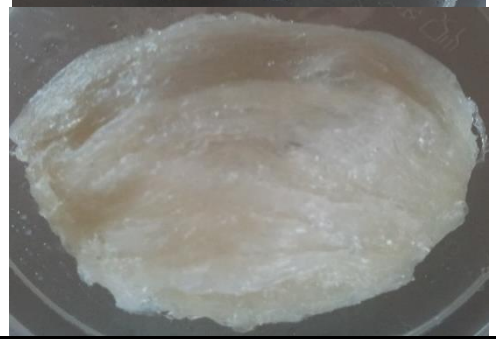
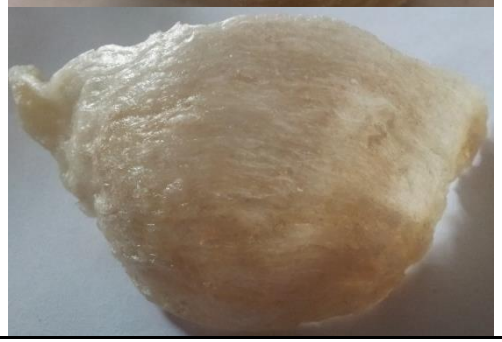
Mean values ± standard deviation (n = 3 replications) within the same column with the same letter are not significantly different (p>0.05).

Table 4.5 Change of colour parameters of continuous and intermittent IR-UVC assisted dried compared to fresh edible bird's nest.

Drying methods	Change of Colour Parameters			Total Colour Change, ΔE
	ΔL^*	Δa^*	Δb^*	
Fresh Edible Bird's Nest	0.00 ± 0.16^a	0.00 ± 0.21^a	0.00 ± 0.08^a	0.00 ± 0.13^a
Fan Drying at 27°C, 29.5% RH and α = 1.00	22.90 ± 1.14^b	0.00 ± 2.33^a	20.95 ± 0.98^c	31.04 ± 2.24^b
Hot Air Drying at 80°C, 8.3% RH and α = 1.00	17.20 ± 1.98^b	-0.10 ± 0.06^a	13.65 ± 0.59^d	21.96 ± 1.47^c
IR assisted drying at 25°C, 29.5% RH and α = 1.00	23.70 ± 0.25^b	-0.10 ± 0.17^a	24.10 ± 1.63^c	33.84 ± 3.38^b
UVC assisted drying at 25°C, 29.3% RH and α = 1.00	23.40 ± 0.40^b	0.00 ± 0.09^a	23.60 ± 2.11^c	33.27 ± 1.00^b
IR-UVC assisted drying at 40°C and 16.5% RH				
α = 0.20	1.80 ± 0.86^a	0.00 ± 1.08^a	5.45 ± 1.24^a	5.07 ± 4.10^a
α = 0.33	3.00 ± 0.15^a	-0.10 ± 1.27^a	5.95 ± 1.03^a	6.66 ± 3.57^a
α = 0.67	2.60 ± 0.78^a	0.00 ± 0.15^a	4.35 ± 2.23^a	5.74 ± 5.20^a
α = 1.00 (Continuous)	8.80 ± 0.23^c	-0.10 ± 0.09^b	8.75 ± 1.99^b	16.55 ± 5.46^c
IR-UVC assisted drying at 25°C and 27.5% RH				
α = 0.20	0.20 ± 2.24^a	0.00 ± 1.21^a	5.55 ± 1.23^a	5.55 ± 1.03^a
α = 0.33	3.60 ± 2.11^a	-0.10 ± 0.45^a	6.85 ± 0.96^a	7.74 ± 0.69^a
α = 0.67	7.10 ± 3.00^c	0.00 ± 0.81^a	9.35 ± 1.33^b	11.74 ± 6.53^a
α = 1.00 (Continuous)	1.70 ± 0.36^a	0.00 ± 0.70^a	10.65 ± 0.69^b	19.96 ± 1.76^c

Mean values ± standard deviation (n = 3 replications) within same column with the same letter are not significantly different (p >0.05).

Figure 4.10 Colour change of edible bird's nests from same origin but different drying methods.

Drying Method	Observation on Colour Change of Edible Bird's Nest		
	Before Drying	After Drying	After One Month of Storage
Fan assisted drying at 27°C and 29.5% RH			
Hot air drying at 80°C and 8.3% RH			
IR-UVC assisted drying at 40°C and 16.5% RH			

Besides, Umesh-Hebbar and Rastogi (2001) claimed that IR treatment with intermittency resulted in a short drying time and the lowest product colour change. During the application of intermittent infrared radiation, wherein the period of heating of the material followed by cooling, intense displacement of the moisture from the core towards to the surface could be achieved. Consequently, higher rate of mass transfer could be obtained as compared with continuous infrared treatment whilst the product was uniformly heated, giving better quality characteristics. This result is consistent with the findings reported by Chou et al. (2000), Chua et al. (2001a) and Ginzburg (1969). However, increasing intermittency (α>0.75) would substantially increase the total drying time which may not be suitable to some products. Longer tempering period may also cause the moisture reabsorption and quality degradation. Hence, intermittency (α) should be carefully selected in order to obtain optimum product quality (Chandan et al., 2014).

On the other hand, the colour of dried samples obtained by intermittent IR-UVC assisted drying of EBN (IR-UVC25 and IR-UVC40), at α = 0.20, 0.33 and 0.67, respectively, did not change significantly (*p>0.05)* and remained almost the same as the fresh EBN even after 1-month storage as shown in Figure 4.10. However, the colour changes are mainly contributed by the *L** and *b** colour parameters, which increased significantly *(p<0.05)* in sample FAN27 after one month of storage, revealing that Maillard reaction has been triggered and caused the formation of brown pigments. The significant colour change that observed in FAN27 was mainly contributed by the increased moisture content and water activity of the sample. After 1 month storage, the water activity of FAN27 dried sample increased from 0.50 to 0.62, which equivalent to 24% increment of water activity in the same sample. The likelihood of Maillard browning reactions occurred on the sample increases as the water activity increases, reaching a maximum at water activity between 0.6 and 0.7 (Martins et al., 2001).

4.2.2 Moisture reabsorption ability and shrinkage

Water sorption properties such as dry basis holding capacity and water reabsorption capacity are vital parameters in determining the rehydration capacity, shrinkage and the stability of final dried product during storage. Table 4.6 shows the moisture reabsorption capacity and shrinkage of dried EBN that are obtained from various drying trials. Moisture reabsorption characteristics are employed as a parameter to determine quality because these are indicative of degree of alterations occurring during processing (drying and rehydration). This theory is also applicable to EBN as well. Degree of moisture reabsorption is dependent on processing conditions, sample preparation, sample composition and extent of the structural and chemical disruption induced by drying. The moisture reabsorption process depends on structural changes in vegetal tissues and cells of food material during drying, which produces shrinkage and food structure collapse, and reduces the water absorption capacity (Krokida et al., 2003).

As shown in Table 4.6, dry basis holding capacity (DHC) and water absorption holding capacity (WAC) of hot air dried samples are significantly *($p<0.05$)* lower than fan assisted dried samples. The lower shrinkage ratio indicates that higher degree of shrinkage in the dried EBN samples as compared to fresh sample. In turn, the moisture reabsorption ability of hot air dried samples is significantly *($p<0.05$)* lower and the shrinkage of hot air dried samples is significantly *($p<0.05$)* more severe than fan assisted dried samples. Apparently, the heat treatment and drying conditions have significantly altered the moisture reabsorption ability of dried EBN products. At first, shrinkage causes changes in the shape of the product and these changes are due to the stresses developed while water is being removed from the material. The EBN sample shrunk extensively reveals a significant reduction in the number and size of the pores. It also affects the product quality by reducing its wettability, changing its texture and reducing the absorption ability (Witrowa-Rajchert and Rzaca, 2009).

Table 4.6 Corresponding dry basis holding capacity (DHC) index/expansion coefficient, water absorption capacity (WAC) index, moisture reabsorption ability (RA) and shrinkage of dried edible bird's nest in accordance with drying conditions.

Drying Method	Dry Basis Holding Capacity, DHC	Water Absorption Capacity, WAC	Moisture Reabsorption Ability, RA	Shrinkage
Fan Drying at 27°C and 29.5% RH	0.863 ± 0.263^{a}	0.799 ± 0.394^{a}	0.690 ± 0.128^{a}	0.890 ± 0.352^{a}
Hot Air Drying at 80°C and 29.3%RH	0.686 ± 0.185^{b}	0.512 ± 0.147^{b}	0.351 ± 0.084^{b}	0.500 ± 0.216^{b}
IR assisted drying at 25°C, 29.5% RH and α = 1.00	0.806 ± 0.126^{a}	0.796 ± 0.251^{a}	0.642 ± 0.117^{a}	0.725 ± 0.018^{a}
UVC assisted drying at 25°C, 29.3% RH and α = 1.00	0.800 ± 0.309^{a}	0.772 ± 0.108^{a}	0.615 ± 0.291^{a}	0.699 ± 0.026^{a}
IR-UVC assisted drying at 40°C and 16.5% RH				
α = 0.20	0.997 ± 0.105^{ac}	0.945 ± 0.199^{ac}	0.942 ± 0.250^{ac}	0.870 ± 0.179^{ac}
α = 0.33	0.899 ± 0.211^{ac}	0.956 ± 0.355^{ac}	0.859 ± 0.184^{ac}	0.840 ± 0.238^{ac}
α = 0.67	0.864 ± 0.237^{ac}	0.899 ± 0.316^{ac}	0.777 ± 0.401^{ac}	0.830 ± 0.154^{ac}
α = 1.00 (Continuous)	0.812 ± 0.262^{ad}	0.866 ± 0.231^{ad}	0.703 ± 0.265^{ad}	0.820 ± 0.333^{ad}
IR-UVC assisted drying at 25°C and 27.5% RH				
α = 0.20	0.995 ± 0.159^{ac}	0.997 ± 0.078^{ac}	0.992 ± 0.436^{ac}	0.890 ± 0.421^{ac}
α = 0.33	0.926 ± 0.133^{ac}	0.912 ± 0.124^{ac}	0.845 ± 0.255^{ac}	0.870 ± 0.138^{ac}
α = 0.67	0.912 ± 0.226^{ac}	0.936 ± 0.268^{ac}	0.854 ± 0.320^{ac}	0.820 ± 0.200^{ac}
α = 1.00 (Continuous)	0.874 ± 0.350^{ad}	0.861 ± 0.334^{ad}	0.753 ± 0.299^{ad}	0.790 ± 0.186^{ad}

Mean values ± standard deviation (n = 3 replications) within same column with the same letter are not significantly different (p >0.05).

On the other hand, the moisture reabsorption ability of IR-UVC assisted dried samples is significantly *($p<0.05$)* higher than hot air dried samples. Samples dried by IR-UVC assisted drying show higher moisture reabsorption ability even after longer drying periods compared to hot air drying. This proves that removal of water at low temperature condition is less destructive to the tissue and hence retains the integrity of the cell walls. As a result, water molecules are held at higher tension; thus reduce water activity, increase moisture reabsorption ability and minimize the shrinkage of dried EBN (Sosle et al., 2003).

It should be noted that water absorption capacity (WAC) and moisture reabsorption ability (RA) indexes vary in the same manner due to only slight changes in DHC values. At higher drying temperatures (80°C), water absorption capacity (WAC) and moisture reabsorption ability (RA) indexes decrease much more rapidly, but changes linearly with moisture content. These results contradict with other food products such as chestnuts (Moreira et al., 2008) and strawberry (Meda and Ratti, 2005) where food sample dried by high temperature over a shorter duration of time shows lesser structural damage and negligible leaching, thus indicating higher values of WAC and RA indexes.

Moreover, the moisture reabsorption ability of intermittent IR-UVC assisted dried samples is significantly *($p<0.05$)* higher than continuous IR-UVC assisted dried samples. In terms of shrinkage, continuous IR-UVC assisted dried samples are significantly *($p<0.05$)* shrunk when compared with intermittent IR-UVC dried samples. This could be due to intermittent drying gives a much lower final moisture content and consequently lower water activity value, resulted in better moisture reabsorption ability and negligible leaching of soluble compounds (Moreira et al., 2008; Ong and Law, 2011).

4.3 Biochemical Properties

4.3.1 Reduction of nitrite

In this study, a novel IR-UVC assisted drying system was developed to improve moisture removal while the UVC system is capable to retard microbial growth (Dial et al., 2012). The results were compared with samples dried by fan assisted drying (conventional method). The results obtained in this study revealed that the nitrite content of EBN dried by fan assisted drying is higher than 30 ppm, which is the restriction control on nitrite content of dried EBN by WHO Standard.

Table 4.7 Nitrite and nitrate contents of edible bird's nest that dried by different drying methods as compared with fan assisted dried (industrial sample).

Drying Method	Nitrite Concentration, X_1 (ppm)	Nitrate Concentration, X_2 (ppm)
Fresh edible bird's nest	40.31 ± 0.64^{e}	329.58 ± 0.34^{e}
Fan Drying at 27°C and 29.5% RH	36.95 ± 1.23^{a}	245.86 ± 1.67^{a}
Hot Air Drying at 80°C and 8.3% RH	7.25 ± 3.56^{bc}	92.24 ± 13.00^{b}
IR assisted drying at 25°C, 29.5% RH and α = 1.00	10.99 ± 1.68^{b}	94.21 ± 2.10^{b}
UVC assisted drying at 25°C, 29.3% RH and α = 1.00	9.26 ± 0.13^{b}	81.20 ± 9.48^{d}
IR-UVC assisted drying at 40°C and 16.5% RH		
α = 0.20	7.82 ± 1.03^{c}	88.57 ± 2.67^{c}
α = 0.33	6.59 ± 3.62^{c}	89.60 ± 11.85^{c}
α = 0.67	6.57 ± 2.58^{c}	84.25 ± 20.84^{c}
α = 1.00 (Continuous)	5.31 ± 1.02^{d}	77.78 ± 10.57^{f}
IR-UVC assisted drying at 25°C and 27.5% RH		
α = 0.20	7.25 ± 1.11^{c}	90.95 ± 1.10^{bc}
α = 0.33	7.53 ± 4.64^{c}	84.87 ± 13.67^{c}
α = 0.67	6.64 ± 4.07^{c}	86.74 ± 12.69^{c}
α = 1.00 (Continuous)	5.70 ± 1.58^{d}	81.25 ± 16.60^{d}

Mean values ± standard deviation (n = 3 replications) within same column with the same letter are not significantly different *(p>0.05).*

**Note: Restriction control on nitrite content of dried edible bird's nest by WHO Standard is 30ppm.*

Table 4.7 shows that nitrite content of fan assisted dried samples is significantly *($p<0.05$)* higher than IR-UVC assisted dried samples. Chan (2013) reported proteomic analysis on EBN which shows that a protein has been identified as the periplasmic nitrate reductase originated from nitrogen fixing bacteria (Nitrobacteria). It might be due to the nitrobacteria multiplication during long duration of drying, which further induced the increase in nitrite content as the water activity is well above 0.6 (water activity of fan assisted sample = 0.62) which is favourable to microbial growth. Water activity determines the lower limit of "available" water for microbial growth. Since bacteria, yeast and moulds require a certain amount of "available" water to support growth, it is essential to maintain the water activity of the dried sample below 0.60. This "desert-like" condition creates an osmotic imbalance between the microorganisms and the local environment. Consequently, the microbes cannot grow and its numbers will decline until it eventually dies (Martins et al., 2001).

Moreover, Table 4.7 also shown that the nitrite content of IR-UVC assisted dried samples reduced significantly *($p<0.05$)* as compared to IR or UVC assisted dried samples. This could be due to prolonged UVC exposure during drying and infrared drying helped to speed up the drying process, which further inhibited the growth of nitrobacteria. According to Riadh et al. (2015), UVC can give rapid and uniform heating and IR radiation can penetrate directly into the inner layer of the material without heating the surrounding air, hence lower the drying time. Besides, high quality dried products, intermittent energy source, easy control of the process parameters, uniform temperature distribution, and clean operational environment, as well as space savings can be achieved by infrared drying as compared to other drying techniques (Riadh et al., 2015; Sakai and Hanzawa, 1994).

Besides, the installation of a germicidal ultraviolet (UVC) light system in the dryer is able to control the microbial activity. It can be seen in Table 4.7 that the nitrite

content by continuous IR-UVC assisted drying at 40°C has been significantly *(p<0.05)* reduced as compared to intermittent and continuous IR-UVC assisted drying at 25°C. This indicates that prolonged exposure to UVC and IR are effective in retarding microbial growth during the drying process. Holt et al. (1993) stated that Nitrobacteria seem to grow optimally at 25-28°C and at pH 7.6 - 7.8. Hence, IR-UVC assisted drying system at 40°C could effectively retard the nitrobacteria growth as compared to drying temperature at 25°C.

4.3.2 Retention of sialic acid

N-Acetyl-neuraminic acid (also called sialic acid, NANA) content is recognized as a unique indicator for the grades of real EBN. According to literature, five monoses,0.7% $_D$-Mannose (Man), 10.9% D-Galactose (Gal), 7.2% N-Acetyl-$_D$-galactosamine (AcGal), 5.3% N-Acetyl-$_D$-glucosamine (AcGlu), and 9% N-Acetyl-neuraminic acid (also called sialic acid, NANA) that constitute the oligosaccharide chain conjugating to glycoprotein in swiftlet's saliva are present in EBN (Kathan and Weeks, 1969; Ma and Liu, 2012; Yang et al., 2013; Yu et al., 2000). As shown in Tables E1-E3 (Appendix E), the calibration curves of sialic acid are linear in the range from the limit of quantification (LOQ) to 20-100 mg/mL, with highly satisfactory correlation coefficients, R^2 = 0.9966. The intra-day precision fell within the range from 3.3% to 10.6%, whereas the inter day precision gave relative standard deviation (RSD) lower than 6.6%. Recoveries of the method were tested at five different concentration levels of standard monoses, and were within the range from 71.4% to 95.5%.

Sialic acid content in fresh EBN was determined at 99.82 ± 2.06 mg/g dw. It was observed that the sialic acid content in samples decreased after drying, about 5% to 69% with reference to fresh sample, depending on the types of dryer and its operating temperature (Figure 4.11). The sialic acid content by hot air-dried at 80°C was recorded as 42.28 mg sialic acid/ g dry weight. While, the sialic acid

content in continuous IR-UVC assisted-dried EBN at 25°C and 40°C were 83.55 mg sialic acid/g dry weight and 78.32 mg sialic acid/g dry weight, respectively. Generally, relatively low temperature used in IR-UVC assisted continuous drying and intermittent drying had resulted in higher retention of sialic acid in dried samples. The effects of high temperature (above 40°C) on retention of sialic acid content can be seen more clearly from the hot air dried sample (HA80), where the sialic acid content in hot air dried sample decreased significantly ($p<0.05$) with increased temperature. The statistical analysis shows that the sialic acid in EBN that were dried by hot air drying at 80°C was significantly different from the fresh EBN sample. The retention of sialic acid by continuous IR-UVC assisted drying at 25°C and 40°C were 83.9% and 78.7%, respectively; while the retention of sialic acid by hot air drying at 80°C is only 42.5% if compared with the sialic acid content in the fresh sample.

The results depict that loss of sialic acid in EBN during drying is more temperature dependent rather than other factors such as the drying duration, moisture content. The reduction in sialic acid during drying is mainly attributed to the thermal degradation while enzymatic oxidation is insignificant. The noticeable loss of sialic acid content under high drying temperature is the loss of stabilizing activity in molecular structures of sialic acid according to the progression of denaturation of sialic acid molecules. The alteration of sialic acid molecules subjected to relatively extensive heat treatment, is considered to be due to intermolecular aggregation. This change is a combination of endothermic reactions, such as the breakup of hydrogen bonds, and exothermic reactions, such as protein aggregation and the breakup of hydrophobic interactions. The endothermic nature is an indication of the very large contribution of the disruption of hydrogen bonds. Differences in sialic acid denaturation temperature were controlled more by the non-polar hydrophobic interactions and a type of "cooperativity" between the polar and the non-polar groups (Arntfield and Murray,

1981; Privalov and Pfeil, 1979). Under severe heat treatments>60°C, the incident formation of structural alteration or association becomes difficult because of partial degradation of sialic acid molecules. The enthalpy of denaturation was dependent upon the temperature of denaturation in that the contribution due to the breakup of hydrophobic interactions is maximum around 60-80°C (Arntfield and Murray, 1981; Department of Veterinary Services, 2009; Khalifa et al., 1985; Shah and Shukla, 1975).

Interestingly, sialic acid content in sample dried by intermittent IR-UVC assisted drying is significantly ($p<0.05$) higher than sample dried by continuous IR-UVC assisted drying, fan assisted drying and hot air drying. The results reveal that greater retention of sialic acid content in dried EBN can be achieved by IR-UVC assisted intermittent drying. This could be due to the shorter heating period on samples by cutting off the heat flux intermittently and hence minimize the thermal degradation of sialic acid; however, excessive drying time may trigger enzymatic oxidation. In contrast, drying at high temperature may significantly shorten the drying time and hence reduce the enzymatic oxidation but may cause severe thermal degradation (Chin and Law, 2010).

Consequently, increasing intermittency, α>0.75 would substantially increase the total drying time which may not be suitable to some products. Longer tempering period may also cause rehydration and quality degradation. Hence, intermittency should be carefully selected in order to obtain optimum product quality (Chandan et al., 2014). Apparently, IR-UVC assisted drying takes advantage of their ability to generate dehumidified air at low temperature intermittently, which allows rapid effective drying at low temperature and thus better preservation of sialic acid. The results suggest that drying temperature (<40°C) is sufficiently low to minimize the thermal destruction of sialic acid presents in the EBN and thus better preservation of sialic acid.

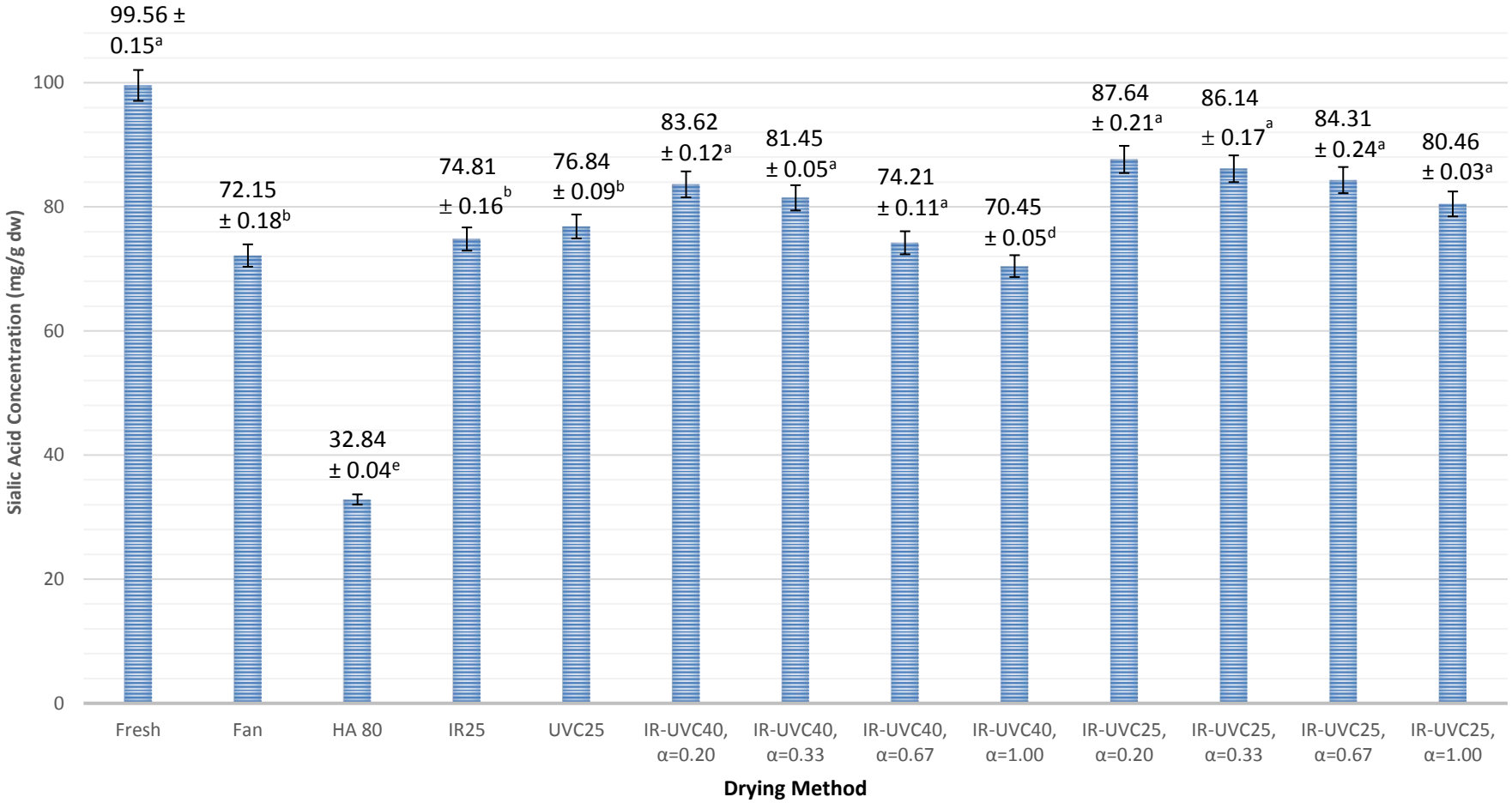


Figure 4.11 Concentration of sialic acid, NANA in dried edible bird's nest samples from various drying experiments. Vertical bars indicate standard error and values marked by the same letter are not significantly different (p>0.05).

4.3.3 Retention of antioxidant

The antioxidant activities from EBN extract are mainly attributed to the amino acids such as cysteine, methionine, histidine, tryptophan and lysine (Guo et al., 2006; Noraini, 2012; Nurfatin, 2014). Two essential amino acids namely lysine and tryptophan are not usually present in most plant proteins. The antioxidant activity of EBN are contributed by the hydroxyl and carboxyl groups in amino acids. Active oxygen and related species play an important physiological role but may exert toxic effects as well. It could have causative role in heart disease, cancer and aging (Guo et al., 2006). Total antioxidant capacity (TAC) reduces with oxidative stress while the chain breaking antioxidant activity increases antioxidant capacity (Young, 2001). The radical scavenging activity of fresh and dried EBN were investigated based on air-drying temperature, drying method and drying mode as shown in Figures 4.12-4.14.

When compared with natural antioxidants activity in plants and animal products, fresh EBN shows very strong antioxidant activity in inhibiting lipid peroxidation by ABTS, DPPH and FRAP methods. This is in an agreement with the findings reported by Guo et al (2006) and Marcone (2005). In turn, this study shows that EBN extracts are strong radical scavenges and can be considered as good source of natural antioxidants to improve human health. The highest initial radical-scavenging activity determined by ABTS assay for fresh EBN sample was 88.65 ± 0.25 mg TEAC/g dry weight, which is significantly higher ($p<0.05$) than other food sources such as soy protein (Durazzo et al., 2015), cow milk (Durazzo et al., 2015), ginger (An et al., 2016), chokeberry (Samoticha et al., 2016), tomato (Gumusay et al., 2015) and apple (vega-Galvez et al., 2012).

It was observed that antioxidant content in samples decreased after drying, about 5% to 60% with reference to fresh samples, depending on the types of dryer and its operating temperature (Figures 4.12-4.14). By comparing various drying

processes and conditions, the highest antioxidant content preserved in EBN were obtained from samples by continuous IR-UVC assisted drying at 25°C (85.61± 0.09 mg TEAC/g dry weight), followed by continuous IR-UVC assisted drying at 40°C (81.18 ± 0.07 mg TEAC/g dry weight); while the lowest antioxidant activity was the hot air-dried EBN sample at 80°C (34.28 ± 0.14 mg TEAC/g dry weight) and this difference was significant ($p<0.05$). In general, relatively low temperature used in IR-UVC assisted continuous and intermittent drying had resulted in higher retention of antioxidant content in dried samples and no significantly different ($p>0.05$) was observed when compared with fresh samples.

On the other hand, the antioxidant contents of samples dried by fan assisted drying, IR or UVC assisted drying at 25°C are significantly different ($p<0.05$) when compared with fresh samples. This is in agreement with the findings reported by Garau et al. (2007) where long drying and low process temperature caused noticeable reduction in antioxidant activity. The observed profile of ABTS, DPPH and FRAP seems to be related to generation and accumulation of different antioxidant compounds having a varying degree of antioxidant activity developing antagonistic or synergistic effects with themselves or with other constituents of the extract (Zielisnki and Koslowska, 2000). Antioxidant retention by continuous IR-UVC assisted drying at 25°C and 40°C were 96.6% and 91.5%, respectively; while the retention of antioxidant by hot air drying at 80°C was recorded only 38.7%. This shows that the preservation of antioxidant activity in dried EBN sample is highly dependent on the drying temperature during the processing. As shown in the results, the effects of high temperature (above 40°C) on the retention of total antioxidant capacity can be clearly seen from the hot air-dried samples.

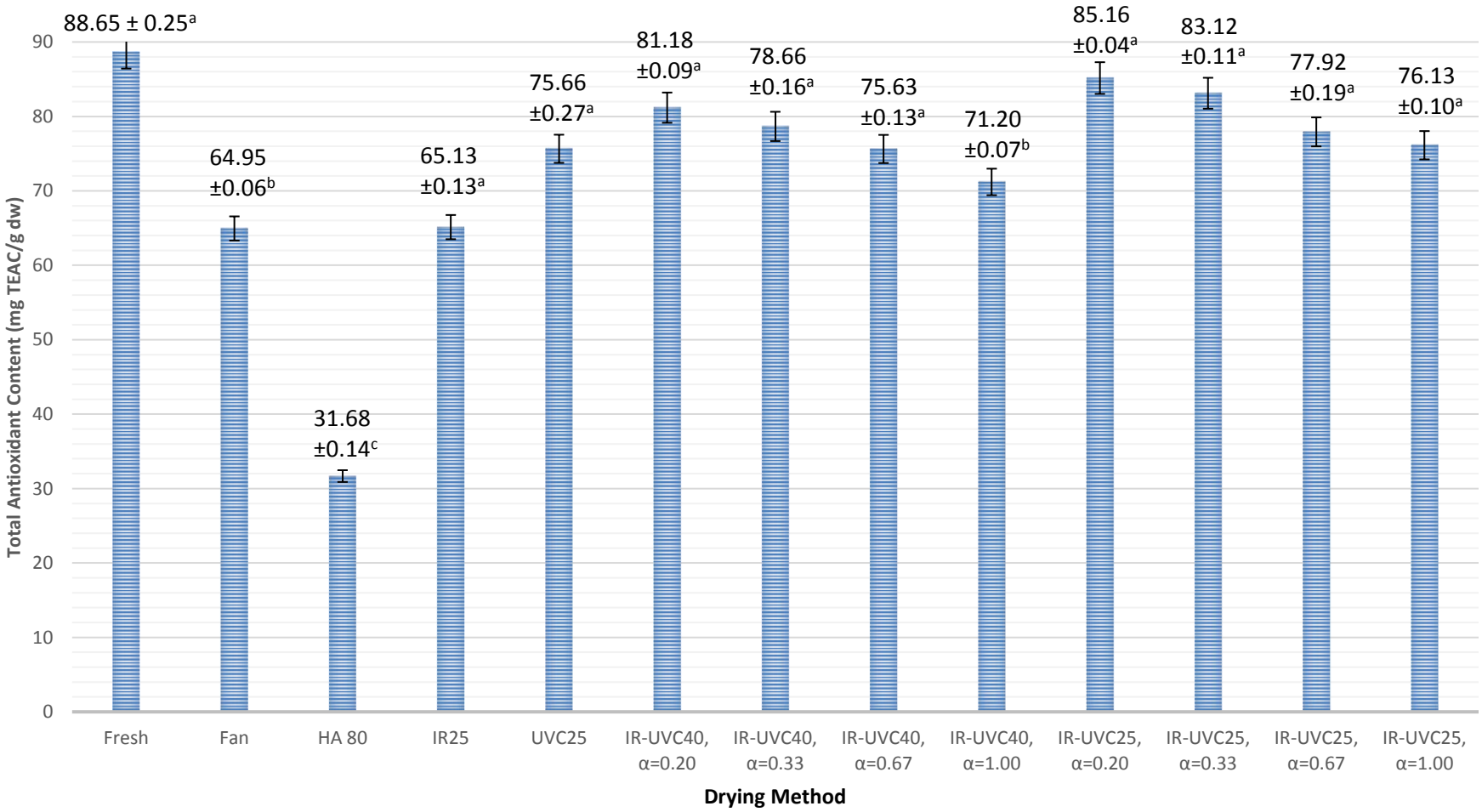


Figure 4.12 Concentration of total antioxidant capacity by ABTS assay in dried edible bird's nest samples from various drying experiments. Vertical bars indicate standard error and values marked by the same letter are not significantly different (p>0.05).

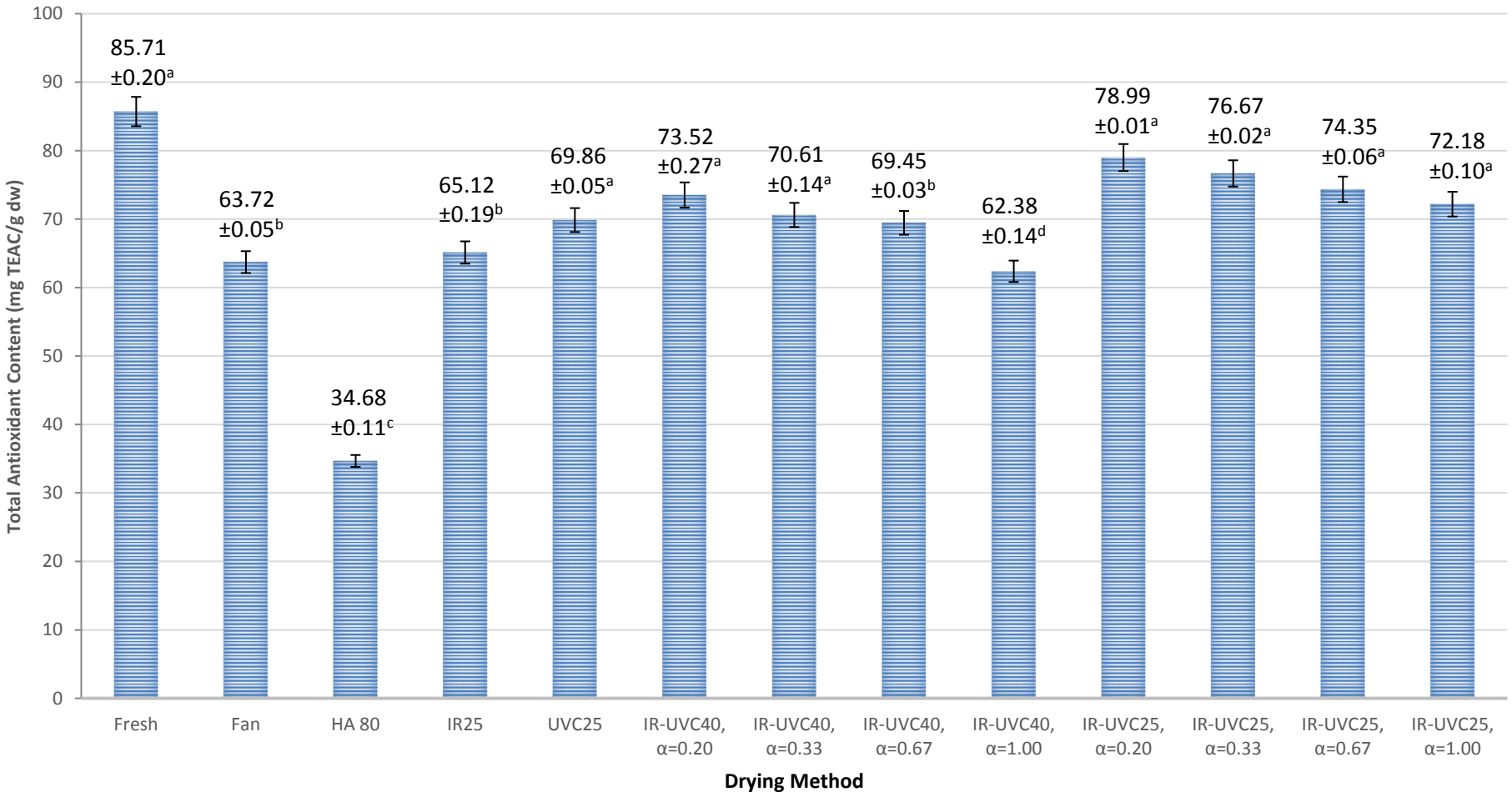


Figure 4.13 Concentration of total antioxidant capacity by DPPH assay in dried edible bird's nest samples from various drying experiments. Vertical bars indicate standard error and values marked by the same letter are not significantly different (p>0.05).

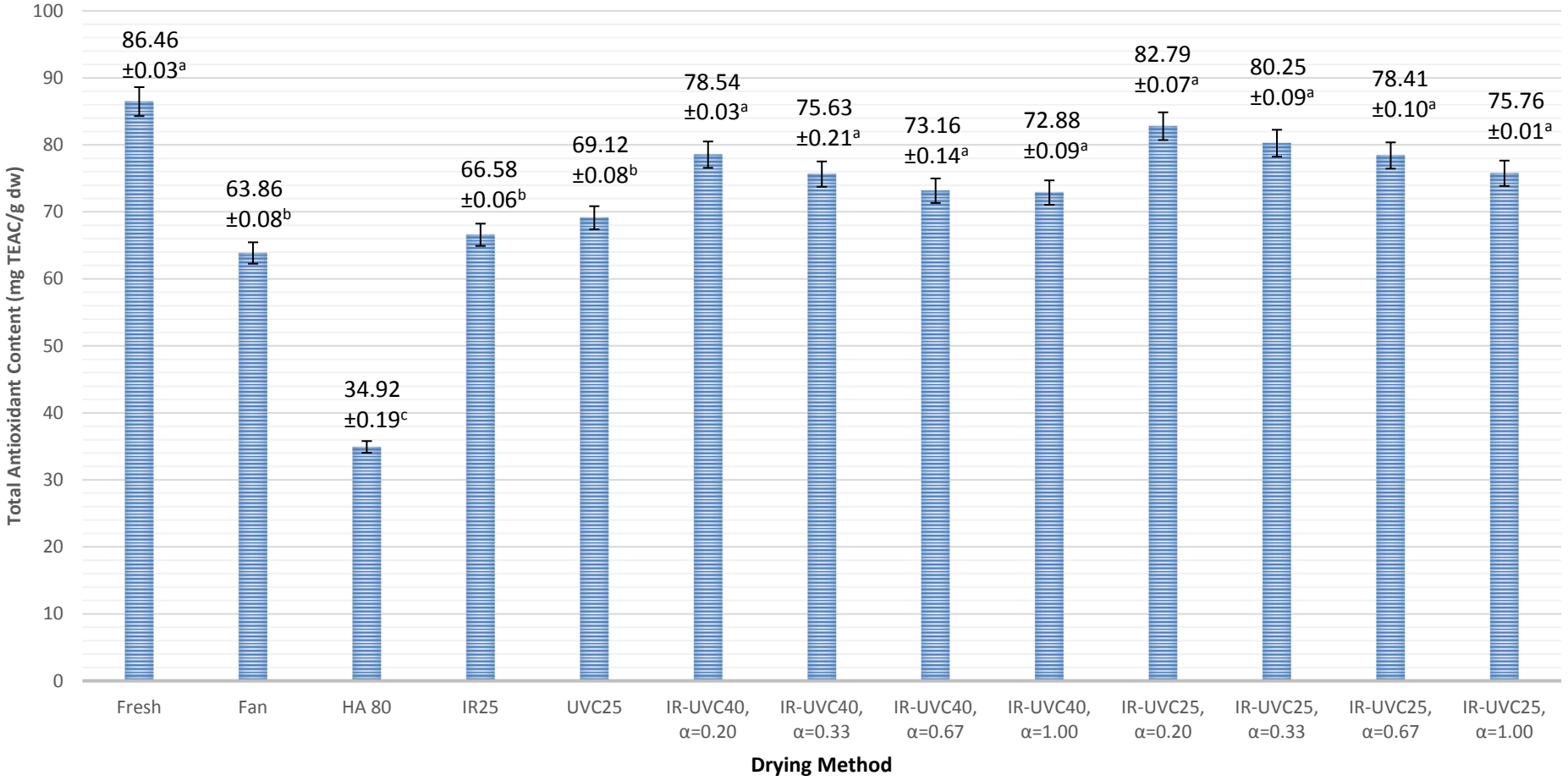


Figure 4.14 Concentration of total antioxidant capacity by FRAP assay in dried edible bird's nest samples from various drying experiments. Vertical bars indicate standard error and values marked by the same letter are not significantly different (p>0.05).

It was observed that total antioxidant capacity in hot air-dried samples decreased with increased drying temperature. The decrease in total antioxidant capacity may be caused by three possible mechanisms, which are the release of bound phenolic compounds, partial degradation of lignin, which could lead to the release of phenolic acids and thermal degradation of the sialic acids. The presence of sialic acid seems to play a vital role irrespective to its position as observed in several antioxidative peptide sequences, which has contributed to its higher radical scavenging potential (Larrauri et al., 1997). The results suggest that drying temperature in IR-UVC assisted dryer at 40°C is sufficiently low to minimize thermal destruction of antioxidant in EBN. It should be noted that this is one of the pioneer study on the antioxidant degradation of EBN by IR-UVC assisted drying.

4.4 Storage Stability of Dried EBN

Undeniably, heat treatment history has great impact on the stability of dried EBN during storage. The effects of drying method and drying mode on moisture content and water activity, colour degradation, prevalence of nitrite and its effect on colour, sialic acid and antioxidant degradation of dried EBN during storage are discussed in the following subsections 4.4.1-4.4.4.

4.4.1 Moisture content and water activity

The moisture content and water activity values of all dried samples after 6-month storage are shown in Tables 4.8 and 4.9. The three different storage methods applied in this study are UV assisted storage cabinet with storage environment control at 27°C and 28.9% RH (method 1), closed plastic container and stored inside the refrigerator at -2°C (method 2) and vacuum packaging (method 3). It can be seen that moisture content and water activity values of all dried samples under storage method 2 increased significantly ($p<0.05$) after 6-month storage. However, the moisture content and water activity values of dried samples stored

under methods 1 and 3 were not significantly different ($p>0.05$). The least increment in moisture content of all dried samples is from storage by vacuum packaging (method 3).

It can be seen from Table 4.8 that moisture content of samples dried using fan assisted drying and intermittent IR-UVC assisted drying at 25°C and 27.5% RH are slightly higher as compared to other samples after 6-month of storage using method 1, with values ranging from 0.12 to 0.16 and 0.11 to 0.12 g water/g dry solid, respectively. Meanwhile, lower moisture content was recorded in samples dried using hot air drying and continuous IR-UVC assisted drying at 40°C and 16.5% RH, with values ranging from 0.11 to 0.12 and 0.10 to 0.15 g water/g dry solid, respectively. This may be due to the dried products by hot air drying and continuous IR-UVC assisted drying with considerable shrinkage and dense structure have resulted in lower moisture reabsorption capacity.

Based on the results shown in Tables 4.8 and 4.9, it could be claimed that the moisture sorption characteristics of the dried products had affected greatly by the drying method and process conditions, which in turn resulted in different moisture gaining rates during storage. This is probably due to the dried products (by fan assisted drying and intermittent IR-UVC assisted drying) that have minimum microstructure changes and less porous structure; thus having better moisture reabsorption as compared to products with considerable shrinkage and dense structure, such as those in hot air drying.

Table 4.8 Moisture content of dried edible bird's nest samples by various drying experiments before and after storage.

Drying method	Moisture content (g water/ g dry solid)						
	Before storage	Method 1* storage	Moisture gained %	Method 2* storage	Moisture gained %	Method 3* storage	Moisture gained %
FAN27	0.12 ± 0.06^{a}	0.16 ± 0.02^{bd}	33.3 %	0.32 ± 0.03^{a}	166.7 %	0.13 ± 0.02^{c}	8.3 %
HA80	0.11 ± 0.03^{a}	0.12 ± 0.04^{ac}	9.09 %	0.22 ± 0.05^{b}	100.0 %	0.12 ± 0.04^{bc}	9.1 %
IR25	0.12 ± 0.07^{a}	0.14 ± 0.01^{c}	16.7 %	0.34 ± 0.01^{ac}	183.3 %	0.13 ± 0.01^{c}	8.3 %
UVC25	0.11 ± 0.02^{a}	0.16 ± 0.01^{bd}	45.5%	0.35 ± 0.02^{ac}	218.2 %	0.12 ± 0.01^{bc}	9.1 %
IR-UVC40, $\alpha = 1.00$	0.11 ± 0.02^{a}	0.13 ± 0.01^{ac}	18.2 %	0.28 ± 0.01^{g}	127.3 %	0.11 ± 0.01^{a}	0.0 %
IR-UVC40, $\alpha = 0.67$	0.11 ± 0.01^{a}	0.14 ± 0.01^{c}	27.3 %	0.32 ± 0.01^{a}	190.9 %	0.11 ± 0.01^{a}	0.0 %
IR-UVC40, $\alpha = 0.33$	0.11 ± 0.03^{a}	0.14 ± 0.02^{c}	27.3 %	0.32 ± 0.01^{a}	190.9 %	0.11 ± 0.02^{a}	0.0 %
IR-UVC40, $\alpha = 0.20$	0.10 ± 0.01^{a}	0.15 ± 0.01^{cd}	50.0 %	0.40 ± 0.01^{e}	300.0 %	0.11 ± 0.01^{a}	10.0 %
IR-UVC25, $\alpha = 1.00$	0.11 ± 0.05^{a}	0.16 ± 0.04^{bd}	45.5 %	0.45 ± 0.03^{f}	309.1 %	0.12 ± 0.04^{bc}	9.1 %
IR-UVC25, $\alpha = 0.67$	0.11 ± 0.04^{a}	0.16 ± 0.03^{bd}	45.5 %	0.47 ± 0.03^{f}	372.3 %	0.12 ± 0.03^{bc}	9.1 %
IR-UVC25, $\alpha = 0.33$	0.11 ± 0.02^{a}	0.17 ± 0.01^{bd}	54.5 %	0.46 ± 0.04^{f}	318.2 %	0.12 ± 0.01^{bc}	9.1 %
IR-UVC25, $\alpha = 0.20$	0.11 ± 0.01^{a}	0.18 ± 0.03^{e}	63.6 %	0.48 ± 0.02^{f}	336.4 %	0.12 ± 0.03^{bc}	9.1 %

Mean values ± standard deviation (n = 3 replications) within same column with the same letter are not significantly different (p>0.05).

**Note: Method 1 is UV assisted storage cabinet with storage environment control at 27°C and 28.9% RH.*
Method 2 is closed plastic container and put into the refrigerator at -2°C.
Method 3 is vacuum packaging.

Table 4.9 Water activity of dried edible bird's nest samples by various drying experiments before and after storage.

Drying method	Water activity, a_w						
	Before storage	Method 1* storage	a_w increment %	Method 2* storage	a_w increment %	Method 3* storage	a_w increment %
FAN27	0.57 ± 0.01^{d}	0.67 ± 0.02^{g}	17.5 %	0.68 ± 0.02^{f}	19.3 %	0.58 ± 0.03^{b}	1.8 %
HA80	0.53 ± 0.02^{b}	0.58 ± 0.03^{cd}	9.43 %	0.61 ± 0.03^{d}	15.1 %	0.54 ± 0.04^{a}	1.9 %
IR25	0.51 ± 0.02^{a}	0.57 ± 0.04^{cd}	11.8 %	0.62 ± 0.04^{d}	21.6 %	0.52 ± 0.05^{a}	2.0 %
UVC25	0.46 ± 0.01^{bc}	0.53 ± 0.01^{a}	15.2%	0.63 ± 0.01^{a}	36.9 %	0.47 ± 0.03^{c}	2.2 %
IR-UVC40, $\alpha = 1.00$	0.47 ± 0.02^{bc}	0.53 ± 0.01^{a}	12.8 %	0.58 ± 0.01^{ab}	23.4 %	0.47 ± 0.02^{c}	0.0 %
IR-UVC40, $\alpha = 0.67$	0.50 ± 0.01^{a}	0.54 ± 0.01^{ab}	8.0 %	0.56 ± 0.01^{b}	12.0 %	0.51 ± 0.01^{a}	2.0 %
IR-UVC40, $\alpha = 0.33$	0.51 ± 0.02^{a}	0.55 ± 0.02^{ac}	7.8 %	0.58 ± 0.02^{ab}	13.7 %	0.51 ± 0.01^{a}	0.0 %
IR-UVC40, $\alpha = 0.20$	0.48 ± 0.01^{bc}	0.56 ± 0.02^{ac}	16.7 %	0.59 ± 0.02^{c}	22.9 %	0.49 ± 0.01^{ac}	2.1 %
IR-UVC25, $\alpha = 1.00$	0.52 ± 0.01^{a}	0.58 ± 0.01^{cd}	11.5 %	0.62 ± 0.01^{d}	19.2 %	0.52 ± 0.01^{a}	0.0 %
IR-UVC25, $\alpha = 0.67$	0.53 ± 0.02^{b}	0.59 ± 0.02^{cd}	11.3 %	0.63 ± 0.02^{d}	18.9 %	0.54 ± 0.01^{b}	1.9 %
IR-UVC25, $\alpha = 0.33$	0.54 ± 0.02^{ab}	0.60 ± 0.02^{e}	11.1 %	0.64 ± 0.02^{e}	18.5 %	0.55 ± 0.02^{a}	1.9 %
IR-UVC25, $\alpha = 0.20$	0.56 ± 0.02^{d}	0.63 ± 0.03^{f}	12.5 %	0.64 ± 0.03^{e}	14.3 %	0.57 ± 0.02^{b}	1.9 %

Mean values ± standard deviation (n = 3 replications) within same column with the same letter are not significantly different (p > 0.05).

**Note: Method 1 is UV assisted storage cabinet with storage environment control at 27°C and 28.9% RH.*
Method 2 is closed plastic container and put into the refrigerator at -2°C.
Method 3 is vacuum packaging.

At first, shrinkage causes changes in the shape of the product. These changes are due to the stresses developed while water is removed from the material. The dried products experienced noticeable shrinkage reveal a significant reduction in the number and size of the pores. It also affects the product quality by reducing its wettability, changing its texture and reducing the adsorption ability (Witrowa-Rajchert and Rzaca, 2009). On the other hand, it was observed that moisture content of all dried samples under cold storage (method 2) increased as well during storage. The increment of moisture content followed the similar trend as in method 1 but recorded a much higher values as compared to all dried samples stored by method 1, which is ranging from 0.56 to 0.68 g water/g dry solid.

In addition, it was observed that water activity values of all dried samples increased (0.55 to 0.65) under both methods 1 and 2, as a result of increased moisture content during storage. Water activity, a_w is a measure of how efficiently the water present can take part in a chemical or physical reactions. Free water in the food is the water available for chemical reactions, to support microbial growth and to act as a transporting medium for compounds. In the bound state, water is not available to participate in these reactions as it is bound by water soluble compounds such as sugar and by the surface effect of the substrate matrix binding. Therefore, when water activity increases, chemical/biochemical reactions (e.g. the Maillard reaction) take place and possibly change the product stability in term of microbiological stability, chemical stability, protein and vitamins content, colour, taste and nutritional values. The product is also stable with respect to lipid oxidation, non-enzymatic browning, enzyme activity, and various microbial parameters at a_w less than 0.3. As a_w increases to more than 0.7, the food product is prone to deteriorate (Martins et al., 2001; Witrowa-Rajchert and Rzaca, 2009).

4.4.2 Prevalence of nitrite and its effect on colour

Table 4.10 shows the nitrite content of dried EBN samples by different drying techniques and stored at different conditions and methods. Based on the results shown in Table 4.10, all nitrite contents are below the allowable limit (30 ppm) set by WHO Standard (MS 2334:2010) and also in line with Food Regulations 1985 (AQSIQ, 2011) as shown in Appendix C (Table AI). After 6-month storage, the increment of nitrite content in all dried EBN samples (except samples dried by fan assisted drying) is not significantly different ($p>0.05$) among the three storage methods. The higher the water activity of the dried sample, the greater increment of nitrite content in all dried samples especially in method 2, which ranges from 6.09 to 38.53 ppm. This is probably due to increment in water activity of fan assisted dried samples to 0.68, where it is vulnerable to various deleterious reactions. As a result, nitrobacteria count increased.

In addition, the change in colour of EBN during storage (i.e. from white to yellowish) has been shown to be related to nitrite content in EBN as shown in Table 4.10. It can be seen from Table 4.10 that the greater increment of nitrite content in the dried sample, the greater colour change could be observed in the dried samples. The highest increment in nitrite content in fan assisted dried sample, which was recorded at 15.10% had induced the greatest colour change at 58.31 % increment. The nitrite content of samples from fan assisted drying is significantly higher $(p<0.05)$ as compared to other samples after 6-month storage by method 1 and 2, with values ranging from 36.95 to 37.01 ppm and 36.95 to 42.53 ppm, respectively. It can also be observed that water activity of dried samples stored using method 1 and 2 (Table 4.9) ranges from 0.53 to 0.68 and 0.58 to 0.69, respectively.

The high water activity in fan assisted dried samples had created a favourable condition for the growth and multiplication of nitrobacteria during storage. As a_w

increases to more than 0.7, the probability of the food product deteriorating increases (Martins et al., 2001; Witrowa-Rajchert and Rzaca, 2009). In turn, the nitrite content and colour change would significantly become higher as compare to those dried samples with lower water activity. On the contrary, conditions in vacuum packaging are not favourable for microbial, yeast and mould growths (Figures 4.17 and 4.20) and it can also be observed that water activity of the vacuum packed dried samples is not significantly different ($p>0.05$). Hence, the least increment in nitrite content and colour change could be observed in all dried samples throughout the three months storage duration. Therefore, it can be recommended that vacuum packaging is a stable storage method for dried EBN samples.

Figures 4.15 - 4.17 (microbial count test) and Figures 4.18 - 4.20 (yeast and mould count test) prove that nitrite content in EBN sample increases with increase in microbial, yeast and mould count in the sample and vice versa. The results also reveal that UV light is definitely an alternative way to reduce the microbial, yeast and mould count therefore reducing the risk associated with the pathogen contamination. On the other hand, from proteomic analysis, a protein was isolated from red EBN which was identified as nitrate reductase deriving from microbes, and this enzyme converts nitrate to nitrite within EBN. The addition of specific inhibitor of nitrate reductase successfully prevented the formation of nitrite in EBN. It is believed that nitrite found in EBN was derived from natural environment, which could be originated from the conversion of nitrate by a nitrate reductase (Chan, 2013).

Table 4.10 Nitrite content gained and colour change of dried samples by various drying experiments before and after 6-month of storage.

Drying method	Before storage		After Method 1* storage		After Method 2* storage		After Method 3* storage	
	Nitrite content (ppm)	Total colour change, ΔE^*	Nitrite gained (%)	Colour change (%)	Nitrite gained (%)	Colour change (%)	Nitrite gained (%)	Colour change (%)
FAN27, $\alpha = 1.00$	36.95 ± 1.23^{a}	31.04 ± 2.24^{b}	0.16 ± 0.12^{a}	19.23 ± 1.02^{a}	15.10 ± 0.18^{a}	78.31 ± 0.36^{a}	0.14 ± 0.01^{a}	2.22 ± 0.07^{a}
HA80, $\alpha = 1.00$	7.25 ± 3.56^{bc}	21.96 ± 1.47^{c}	2.07 ± 0.12^{a}	46.57 ± 0.76^{c}	12.69 ± 0.06^{c}	68.27 ± 0.09^{c}	0.41 ± 0.08^{c}	4.60 ± 0.09^{c}
IR25, $\alpha = 1.00$	10.99 ± 1.68^{e}	33.84 ± 3.38^{b}	2.00 ± 1.01^{g}	40.93 ± 1.20^{c}	6.34 ± 0.37^{g}	44.86 ± 0.16^{d}	0.36 ± 0.07^{g}	4.81 ± 0.10^{a}
UVC25, $\alpha = 1.00$	9.26 ± 0.13^{b}	33.27 ± 1.00^{b}	1.08 ± 0.29^{b}	27.03 ± 0.65^{bc}	3.89 ± 0.02^{bg}	33.20 ± 0.02^{cd}	0.54 ± 0.03^{b}	5.09 ± 0.02^{a}
IR-UVC40, $\alpha = 1.00$	5.31 ± 1.02^{d}	26.55 ± 5.46^{c}	1.88 ± 0.88^{ef}	26.82 ± 1.04^{d}	10.92 ± 0.30^{e}	50.17 ± 0.75^{b}	0.94 ± 0.29^{ef}	6.71 ± 0.03^{b}
IR-UVC40, $\alpha = 0.67$	6.57 ± 2.58^{c}	5.74 ± 5.20^{a}	0.46 ± 0.09^{d}	9.72 ± 2.36^{e}	7.46 ± 0.10^{d}	42.51 ± 1.38^{e}	0.76 ± 0.06^{d}	5.28 ±0.23^{d}
IR-UVC40, $\alpha = 0.33$	6.59 ± 3.62^{c}	6.66 ± 3.57^{a}	0.31 ± 0.03^{d}	4.17 ± 0.98^{e}	6.52 ± 0.06^{c}	36.28 ± 0.09^{e}	1.21 ± 0.04^{cd}	8.46 ± 0.19^{d}
IR-UVC40, $\alpha = 0.20$	7.82 ± 1.03^{c}	5.07 ± 4.10^{a}	1.02 ± 0.19^{c}	25.17 ± 0.25^{e}	4.22 ± 0.13^{c}	30.92 ± 0.64^{e}	0.64 ± 0.08^{c}	4.95±0.26^{de}

Drying method	Before storage		After Method 1* storage		After Method 2* storage		After Method 3* storage	
	Nitrite content (ppm)	Total colour change, ΔE^*	Nitrite gained (%)	Colour change (%)	Nitrite gained (%)	Colour change (%)	Nitrite gained (%)	Colour change (%)
IR-UVC25, $\alpha = 1.00$	5.70 ± 1.58^{d}	29.96 ± 1.76^{cd}	0.88 ± 0.04^{e}	17.11 ± 0.38^{c}	5.96 ± 0.18^{e}	41.75 ± 0.29^{c}	0.53 ± 0.10^{e}	6.38 ± 0.19^{e}
IR-UVC25, $\alpha = 0.67$	6.64 ± 4.07^{c}	11.74 ± 6.53^{e}	0.61 ± 0.06^{d}	12.34 ± 1.11^{f}	3.92 ± 0.38^{e}	36.20 ± 0.44^{f}	0.45 ± 0.05^{d}	4.20 ± 0.15^{f}
IR-UVC25, $\alpha = 0.33$	7.53 ± 4.64^{c}	7.74 ± 0.69^{ae}	0.93 ± 0.04^{c}	19.28 ± 0.43^{eg}	6.37 ± 0.26^{c}	56.51 ± 0.08^{g}	0.40 ± 0.09^{c}	4.49 ± 0.26^{d}
IR-UVC25, $\alpha = 0.20$	7.25 ± 1.11^{c}	5.55 ± 1.08^{a}	0.83 ± 0.10^{c}	18.65 ± 0.55^{e}	7.86 ± 0.77^{b}	48.99 ± 0.49^{e}	0.28 ± 0.01^{c}	2.19 ± 0.17^{d}

Mean values ± standard deviation (n = 3 replications) within same column with the same letter are not significantly different ($p > 0.05$).

**Note: Method 1 is UV assisted storage cabinet with storage environment control at 27°C and 28.9% RH.*
Method 2 is closed plastic container and put into the refrigerator at -2°C.
Method 3 is vacuum packaging.

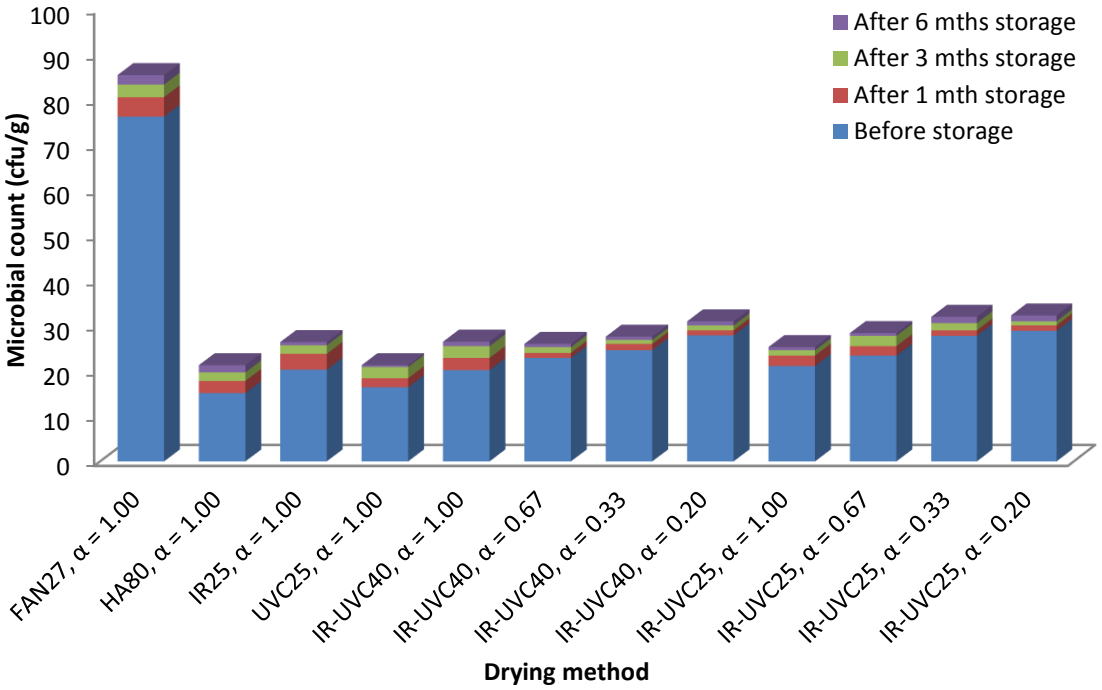


Figure 4.15 Microbial count on all dried samples stored by method 1.

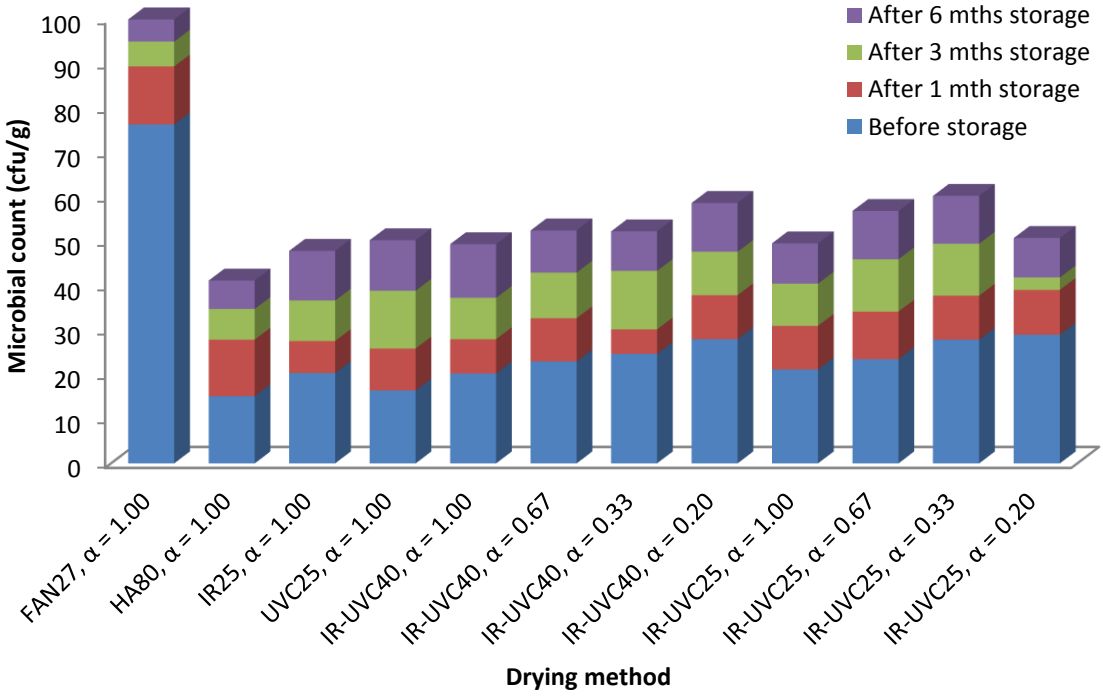


Figure 4.16 Microbial count on all dried samples stored by method 2.

Mean values ± standard deviation (n = 3 replications) within the same column with the same letter are not significantly different (p >0.05).
Note: The tolerance level of microbial count accepted by Malaysia's government is ≤ 2.5 x 10^6 cfu/g (Department of Veterinary Services, 2009).

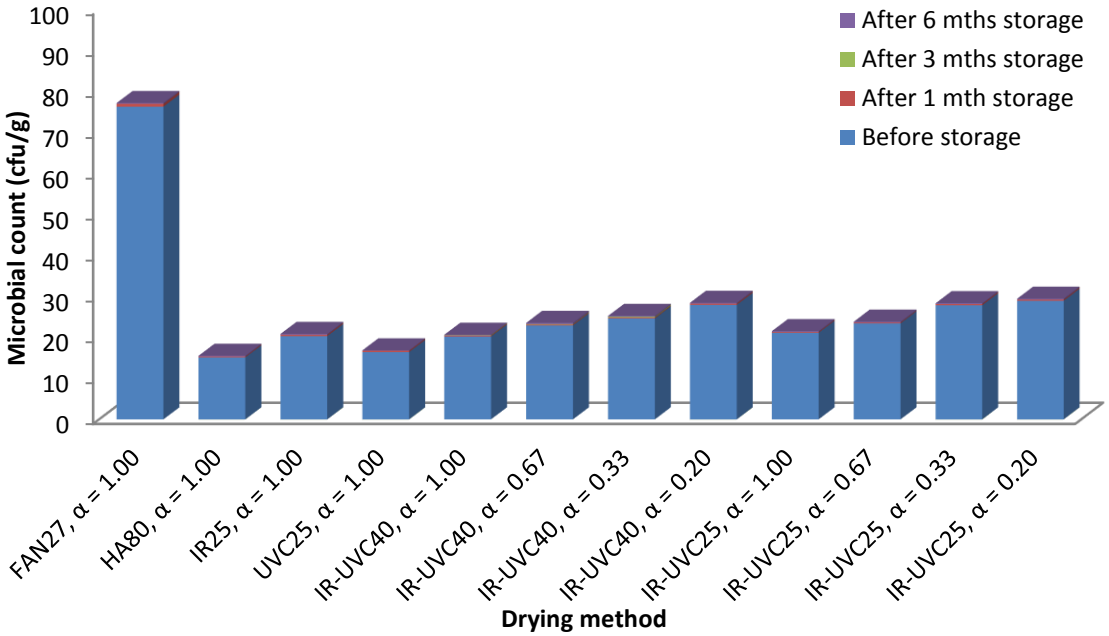


Figure 4.17 Microbial count on all dried samples stored by method 3.

Mean values ± standard deviation (*n* = 3 replications) within the same column with the same letter are not significantly different (*p* >0.05).
Note: The tolerance level of microbial count accepted by Malaysia's government is ≤ 2.5 x 10^6 cfu/g (Department of Veterinary Services, 2009).

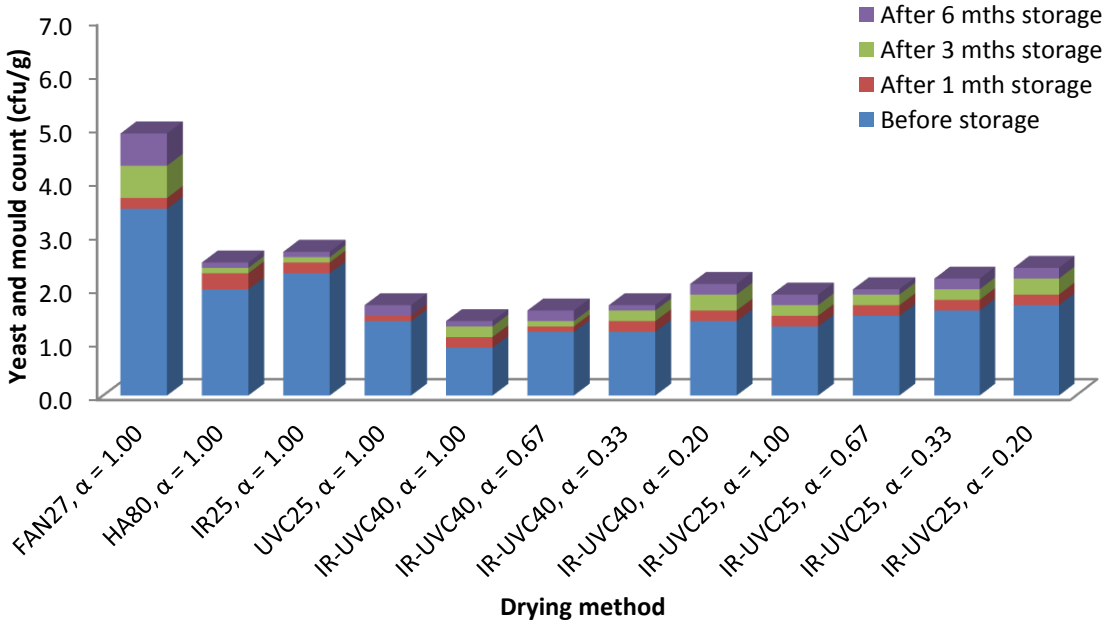


Figure 4.18 Yeast and mould count on all dried samples stored by method 1.

Mean values ± standard deviation (*n* = 3 replications) within the same column with the same letter are not significantly different (*p* >0.05).
Note: The tolerance level of yeast and mould count accepted by Malaysia's government is ≤10 cfu/g (Department of Veterinary Services, 2009).

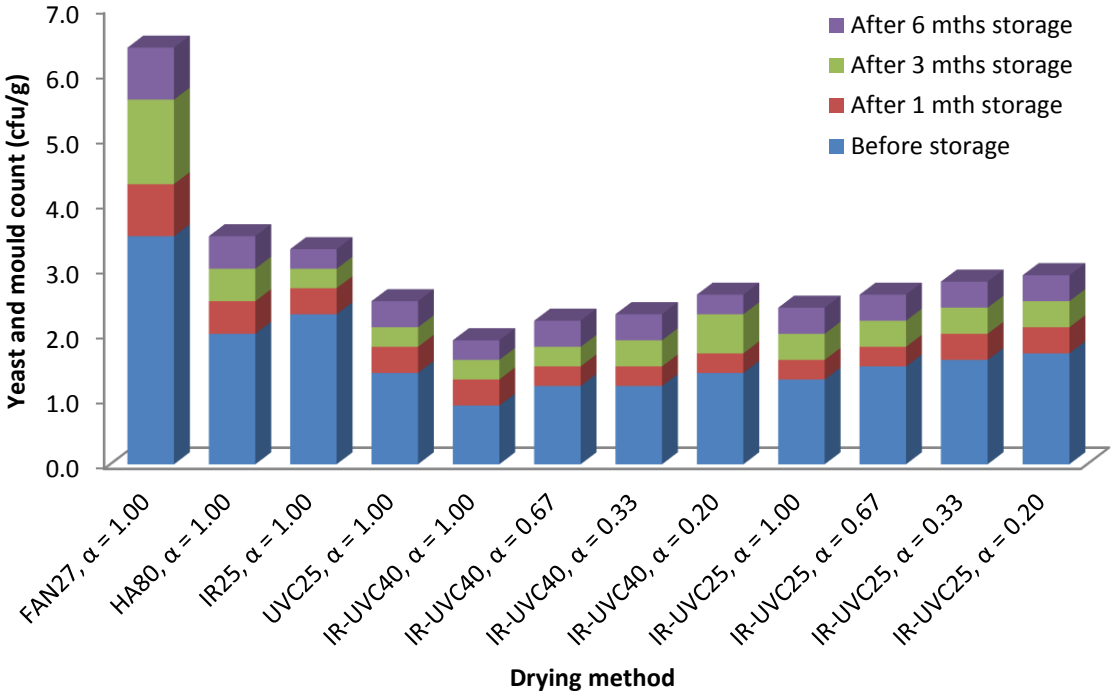


Figure 4.19 Yeast and mould count on all dried samples stored by method 2.

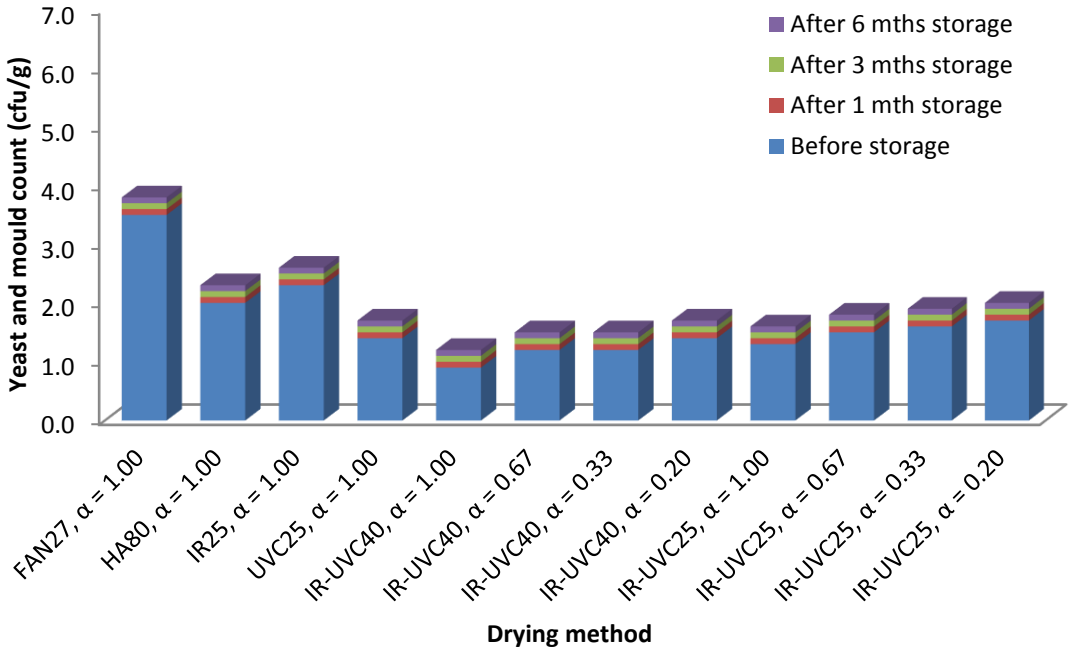


Figure 4.20 Yeast and mould count on all dried samples stored by method 3.

Mean values ± standard deviation (n = 3 replications) within the same column with the same letter are not significantly different (p >0.05).

Note: The tolerance level of yeast and mould count accepted by Malaysia's government is ≤10 cfu/g (Department of Veterinary Services, 2009).

4.4.3 Sialic acid and antioxidant degradation

Tables 4.11 and 4.12 show that sialic acid and antioxidant contents in all samples under these three storage methods (after 6-month) remain high with low percentage loss as compared with the initial concentration. Percentage loss of sialic acid content in samples under these three storage methods ranges from 0.81 % to 3.53 % (method 1), 1.80 % to 7.81 % (method 2) and 0.37 % to 2.56 % (method 3), respectively. In addition, the percentage loss of antioxidant content in samples under similar storage methods ranges from 1.10 % to 2.49 % (method 1), 1.81 % to 8.43 % (method 2) and 0.49 % to 1.86 % (method 3), respectively. The results also show that reduction of sialic acid and antioxidant content in samples is not significant ($p>0.05$) under vacuum packaging. This could be due to storage under vacuum condition that could avoid oxidation of sialic acid and antioxidants in all dried samples regardless of heating treatment history.

On the other hand, reduction of sialic acid and antioxidant in all dried samples are significant ($p<0.05$) under cold storage (method 2) which could be related to the increment in moisture content and browning activities throughout the storage period. During cold storage, crystallization of residual intercellular water is rapidly formed and hence induced structural change of cell compartments in the samples. This could lead to increment in moisture content and water activity of dried EBN and thus induced oxidation of sialic acid and antioxidant contents. Moreover, the slight decrement of sialic acid and antioxidant in all dried samples in method 1 could be due to oxidation of sialic acid and antioxidant which produces brown pigments under ambient condition. The oxidation of bioactive ingredients in dried food is considered as a major deteriorative mechanism and is sensitive to temperature and water activity during storage period (Ong and Law, 2010a).

Table 4.11 Sialic acid content of dried edible bird's nest samples by various drying experiments before and after six months of storage.

Drying method	Sialic acid content (mg/g dry weight)						
	Before storage	After Method 1* storage	Reduction %	After Method 2* storage	Reduction %	After Method 3* storage	Reduction %
FAN27, $\alpha = 1.00$	72.15 ± 0.18^{ac}	70.32 ± 0.14^{a}	2.54 %	69.21 ± 0.07^{a}	4.08 %	71.88 ± 0.01^{a}	0.37 %
HA80, $\alpha = 1.00$	32.84 ± 0.04^{b}	31.68 ± 0.03^{b}	3.53 %	30.44 ± 0.05^{c}	7.31 %	32.00 ± 0.06^{b}	2.56 %
IR25, $\alpha = 1.00$	74.81 ± 0.16^{ac}	73.26 ± 0.09^{a}	2.07 %	72.81 ± 0.04^{b}	2.67 %	74.00 ± 0.07^{a}	1.08 %
UVC25, $\alpha = 1.00$	76.84 ± 0.09^{ac}	75.99 ± 0.11^{a}	1.10 %	75.00 ± 0.04^{b}	2.39 %	76.09 ± 0.03^{a}	0.98 %
IR-UVC40, $\alpha = 1.00$	70.45 ± 0.05^{a}	69.57 ± 0.02^{ac}	1.25 %	68.53 ± 0.01^{a}	2.73 %	69.95 ± 0.04^{ac}	0.71 %
IR-UVC40, $\alpha = 0.67$	74.21 ± 0.11^{ac}	73.10 ± 0.07^{a}	1.50 %	72.01 ± 0.09^{b}	2.96 %	73.62 ± 0.02^{a}	0.80 %
IR-UVC40, $\alpha = 0.33$	81.45 ± 0.05^{d}	80.79 ± 0.05^{d}	0.81 %	79.50 ± 0.10^{b}	2.39 %	80.75 ± 0.01^{d}	0.86 %
IR-UVC40, $\alpha = 0.20$	83.62 ± 0.12^{d}	82.80 ± 0.08^{d}	0.98 %	81.23 ± 0.11^{bd}	2.86 %	82.99 ± 0.01^{d}	0.75 %
IR-UVC25, $\alpha = 1.00$	80.46 ± 0.03^{d}	79.33 ± 0.06^{a}	1.40 %	78.11 ± 0.17^{b}	2.92 %	79.86 ± 0.01^{a}	0.75 %
IR-UVC25, $\alpha = 0.67$	84.31 ± 0.24^{d}	83.52 ± 0.02^{d}	0.94 %	82.79 ± 0.09^{d}	1.80 %	83.76 ± 0.03^{d}	0.65 %
IR-UVC25, $\alpha = 0.33$	86.14 ± 0.17^{cd}	84.99 ± 0.01^{d}	1.34 %	83.90 ± 0.19^{d}	2.60 %	85.63 ± 0.04^{d}	0.59 %
IR-UVC25, $\alpha = 0.20$	87.64 ± 0.21^{cd}	86.38 ± 0.09^{d}	1.44 %	85.72 ± 0.20^{d}	2.19 %	87.01 ± 0.05^{d}	0.72 %

Mean values ± standard deviation (n = 3 replications) within same column with the same letter are not significantly different (p > 0.05).
**Note: Sialic acid content of fresh edible bird's nest was recorded as 99.56 ± 0.15 mg/g dry weight.*

**Note: Method 1 is UV assisted storage cabinet with storage environment control at 27°C and 28.9% RH.*
Method 2 is closed plastic container and put into the refrigerator at -2°C.
Method 3 is vacuum packaging.

Table 4.12 Antioxidant content by ABTS assay of all dried edible bird's nest samples before and after six months of storage.

Drying method	Antioxidant content by ABTS assay (mg/g dry weight)						
	Before storage	After Method 1* storage	Reduction %	After Method 2* storage	Reduction %	After Method 3* storage	Reduction %
FAN27, $\alpha = 1.00$	64.95 ± 0.06^{a}	63.67 ± 0.08^{a}	1.97 %	61.99 ± 0.12^{a}	4.56 %	64.16 ± 0.10^{a}	1.22 %
HA80, $\alpha = 1.00$	31.68 ± 0.04^{b}	30.89 ± 0.02^{b}	2.49 %	29.01 ± 0.08^{c}	8.43 %	31.09 ± 0.06^{c}	1.86 %
IR25, $\alpha = 1.00$	65.13 ± 0.13^{a}	64.01 ± 0.45^{a}	1.12 %	63.42 ± 0.17^{a}	2.63 %	64.82 ± 0.32^{a}	0.48 %
UVC25, $\alpha = 1.00$	75.66 ± 0.27^{b}	74.24 ± 0.20^{c}	1.88 %	73.85 ± 0.16^{b}	1.81 %	74.89 ± 0.08^{a}	1.02 %
IR-UVC40, $\alpha = 1.00$	71.20 ± 0.07^{c}	70.06 ± 0.16^{c}	1.60 %	69.13 ± 0.09^{a}	2.91 %	70.75 ± 0.14^{b}	0.63 %
IR-UVC40, $\alpha = 0.67$	75.63 ± 0.13^{c}	74.32 ± 0.09^{c}	1.73 %	73.09 ± 0.25^{b}	3.36 %	75.08 ± 0.02^{d}	0.73 %
IR-UVC40, $\alpha = 0.33$	78.66 ± 0.16^{c}	77.65 ± 0.05^{c}	1.28 %	75.98 ± 0.08^{b}	3.41 %	78.04 ± 0.01^{d}	0.79 %
IR-UVC40, $\alpha = 0.20$	81.18 ± 0.09^{d}	80.08 ± 0.03^{d}	1.36 %	79.02 ± 0.04^{b}	2.67 %	80.56 ± 0.08^{de}	0.76 %
IR-UVC25, $\alpha = 1.00$	76.13 ± 0.10^{cd}	75.24 ± 0.08^{c}	1.17 %	74.48 ± 0.05^{b}	2.17 %	75.36 ± 0.09^{e}	1.01 %
IR-UVC25, $\alpha = 0.67$	77.92 ± 0.19^{cd}	76.43 ± 1.00^{cd}	1.91 %	75.08 ± 0.12^{b}	3.64 %	77.09 ± 0.26^{f}	1.07 %
IR-UVC25, $\alpha = 0.33$	83.12 ± 0.11^{d}	82.16 ± 0.28^{d}	1.15 %	81.03 ± 0.13^{d}	2.51 %	82.65 ± 0.09^{d}	0.57 %
IR-UVC25, $\alpha = 0.20$	85.16 ± 0.04^{d}	84.22 ± 0.01^{d}	1.10 %	83.07 ± 0.06^{d}	2.45 %	84.38 ± 0.02^{d}	0.92 %

Mean values ± standard deviation (n = 3 replications) within same column with the same letter are not significantly different (p > 0.05).
**Note: Antioxidant content of fresh edible bird's nest was recorded as 86.46 ± 0.03 mg/g dry weight.*

**Note: Method 1 is UV assisted storage cabinet with storage environment control at 27°C and 28.9% RH.*
Method 2 is closed plastic container and put into the refrigerator at -2°C.
Method 3 is vacuum packaging.

4.5 Optimization of IR-UVC Assisted Intermittent Drying

The results reveal that IR-UVC could produce better quality of dehydrated EBN. IR-UVC assisted drying was further tested by performing intermittent drying. Again the results also reveal that the intermittent IR-UVC assisted drying also gave good retention of bio-active ingredients. The optimization of intermittent IR-UVC assisted drying was performed using Response Surface Methodology (RSM). The selection of drying profile and experimental range was discussed in subsection 4.5.1 and the optimization process using response surface methodology was discussed in subsection 4.5.2.

4.5.1 Selection of drying profile and experimental range

Based on the aforementioned experimental results, it can be observed that drying at low temperature is needed for EBN in order to produce dried product with premium quality and better stability during storage. It also discovered that drying time plays an essential role in determining the product quality. Short effective heating time during intermittent drying could avoid undesirable enzymatic reactions that normally occur under long processing time. In turn, intermittent IR-UVC assisted drying at temperature of 40°C and intermittency of 0.20-1.00 (IR-UVC40) allows fast effective drying at mild temperature as compared to other drying methods. The aforementioned experimental results also show that product quality of dehydrated EBN using IR-UVC40 is significantly better ($p<0.05$) as compared to all samples in terms of colour alteration, rehydration capacity and shrinkage, reduction of nitrite and nitrate, retention of sialic acid, retention of antioxidant and storage stability.

4.5.2 Optimization by response surface methodology

An optimization on three drying variables, such as intermittent duration (X_1), intermittent ratio (X_2) and intermittent cycle (X_3) was performed by response surface methodology with the aims to determine the best combination of the

drying variables, in order to avoid excessive long drying period and also to improve product quality and storage stability.

4.5.2.1 Multiple linear regression and analysis of variance (ANOVA)

Multiple regression models that relate the response variables to coded levels of independent variables were developed and analyses of variance (ANOVA) were performed to assess significances of the independent variables on each response variables in the experimental data as shown in Table 4.13. There were six response variables being considered in the present study, such as total drying time (Y_1), total heating time during intermittent (Y_2), total colour change (Y_3), nitrite content change (Y_4), sialic acid content (Y_5) and antioxidant content (Y_6).

Table 4.13 Experimental data for response surface analysis.

Drying variables			Response variables					
X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
390	0.33	2	582	49	7.74	10.56	73.65	79.68
390	0.67	3	1182	49	10.45	4.59	67.43	63.42
620	0.20	2	775	78	5.07	11.52	83.55	84.52
390	0.33	2	582	49	8.56	6.59	77.66	78.41
390	0.33	2	582	49	7.41	6.76	78.74	75.17
390	0.20	3	488	49	5.23	8.95	80.23	81.65
390	0.67	1	1182	49	6.58	3.49	65.13	66.94
620	0.67	2	1879	78	7.59	4.06	70.16	71.52
210	0.33	3	313	26	5.09	6.25	76.34	75.49
620	0.33	3	925	78	12.63	8.53	77.55	76.42
210	0.67	2	636	26	4.63	4.31	70.26	69.58
210	0.33	1	313	26	4.52	11.36	84.88	85.47
390	0.33	2	582	49	4.21	7.94	76.59	79.42
620	0.33	1	925	78	9.52	8.46	85.14	84.35
390	0.33	2	582	49	6.58	8.79	74.56	72.16
390	0.20	1	488	49	8.88	11.55	81.48	84.29
210	0.20	2	263	26	14.23	14.88	84.97	86.70

Note: X_1 = Intermittent duration (min); X_2 = Intermittent ratio, α ; X_3 = Intermittent cycle; Y_1 = total drying time (min), Y_2 = total heating time during intermittent (min), Y_3 = total colour change, Y_4 = nitrite content change (ppm), Y_5 = sialic acid content (mg/g) and Y_6 = antioxidant content (mg/g)

From response surface analysis, intermittent duration (X_1), intermittent ratio (X_2) and intermittent cycle (X_3) significantly *($p<0.05$),* at high confidence level (90-95%) affected the total drying time (Y_1), total heating time during intermittent (Y_2), and total colour change (Y_3). Results indicate that the duration and cycle number of tempering period are important factors in determining total drying time and effective heating time in intermittent IR-UVC assisted drying. On top of that, intermittent duration (X_1) and intermittent ratio (X_2) significantly *($p<0.05$)*, at high confidence level (90%-95%) affected the nitrite content change (Y_4). This is because the extensive long tempering period may prolong drying time and consequently results in growth and multiplication of nitrobacteria. Nonetheless, sialic acid content (Y_5) and antioxidant content (Y_6) are not significantly influenced by all the drying variables ($p>0.10$). In accordance with the experimental results in previous sections, Y_3, Y_5 and Y_6 are more temperature dependent rather than time dependent.

By fitting experimental data from Table 4.13 to mathematical models in the form of equation [3.24], response surface models were developed to further analyses the interrelationship among the variables. Table 4.14 shows the results of analyses of variance (ANOVA) on selected response surface models, in terms of linear, quadratic or interaction, and respective residual variances for all response variables in Table 4.15. It was observed that polynomial models for total drying time (Y_1), total heating time during intermittent (Y_2), total colour change (Y_3) and nitrite content change (Y_4) are statistically significant ($p<0.0001$); while, models for sialic acid content (Y_5) and antioxidant content (Y_6) are insignificant ($p>0.10$) probably due to noise. Quadratic term significantly influences the significance of the models for total drying time (Y_1) and total colour change (Y_3); while, the linear term contributed substantially to the total heating time during intermittent (Y_2) and nitrite content change (Y_4).

Table 4.14 Analysis of variance (ANOVA) showing significances of drying variables on all the response variables in this study.

Drying variables	F value					
	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
X_1	423.80[a]	6.366 x 10^7[a]	2.85[c]	0.52[b]	1.51[c]	2.06[c]
X_2	1856.50[a]	6.366 x 10^7[a]	0.76[c]	3.43[b]	4.26[c]	5.48[c]
X_3	1595.11[a]	6.366 x 10^7[a]	0.60[c]	1.66[b]	2.71[c]	7.10[c]

[a]*Significant at 99.99% level of confidence (p<0.0001)*
[b]*Significant at 95% level of confidence (p<0.05)*
[c]*Not significant (p>0.10)*

Table 4.15 Analysis of variance (ANOVA) showing significances of response surface models on all the response variables in this study.

	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
Sum of Square						
Model	20810[a]	40658[a]	114.20[c]	31.20[b]	475.16[c]	631.40[c]
Linear	20810[c]	40658[a]	114.20[c]	31.20[b]	118.42[c]	90.84[c]
Interaction	43444.13[c]	89573.18[c]	89.84[c]	19.18[c]	105.92[c]	85.23[c]
Quadratic	4775.23[a]	8411.39[c]	85.80[c]	7.19[c]	29.44[c]	20.86[c]
Residual	4775.24	8411.15	133.88	41.82	136.34	132.64
Lack-of-fit	4775.23[c]	8411.14[c]	122.82[c]	31.20[c]	118.42[c]	90.84[c]
Pure error	0.0	0.0	11.06	10.63	17.92	41.80
R^2	0.9982	0.9980	0.9143	0.9378	0.9770	0.9624
CV%	3.62	3.08	1.52	2.00	4.21	4.13
Adeq Precision	78.69	79.84	47.16	10.01	11.44	14.14

[a]*Significant at 99.99% level of confidence (p<0.0001)*
[b]*Significant at 95% level of confidence (p<0.05)*
[c]*Not significant (p>0.10)*

Nonetheless, all models show insignificant lack-of-fit ($p>0.1$) which depicts that all the models could represent the experimental data adequately. Generally, the discrepancy between the observed value and the fitted value could represent by the calculated residual variance, which includes two factors: lack-of-fit and pure experimental error. Lack-of-fit variance is a measure of error due to deficiency in model and can be employed to test if the model can fit the experimental data well. In other words, the lack-of-fit term is insignificant then the model is adequate to represent data in the experimental domain. Whereas, the pure experimental error variance is calculated by considering variation between observations at the same experimental conditions run in random sequence. The variability of the observations within each treatment could be reflected by the pure error (Montgomery, 2001).

Furthermore, it could be observed that high coefficient of determination, R^2 (0.9143-0.9982) and low coefficient of variation percentage, CV% (1.52-8.15) appear in response surface models for all the response variables (Y_1 – Y_7), indicating that the models developed for these responses appeared to be adequate. Besides, insignificant models still could be used to navigate the design space since the signal-to-noise ratio was found to be satisfactorily high (adequate precision > 4). According to Montgomery (2001), the high value in signal-to-noise ratio would indicate that the observing variation could be closely related to the underlying uncertainty of the fitted model. In overall, the results in present study are in good agreement with other studies on optimization of intermittent drying using response surface methodology (RSM) (Erbay and Icier, 2009; Sumic et al., 2016; Thirugnanasambandham and Sivakumar, 2015; Wang et al., 2016).

4.5.2.2 Response surface analysis

Table 4.16 shows the estimated regression coefficients for response surface models that were generated based on the significant terms in each response.

Basically, quadratic model was developed for total drying time (Y_1) and total colour change (Y_3); while, the linear term was developed for total heating time during intermittent (Y_2), nitrite content change (Y_4), sialic acid content (Y_5) and antioxidant content (Y_6).

Table 4.16 Regression coefficients (based on coded data) of response surface models that representing relationship of the drying variables and response variables.

	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
Model type	Quadratic	Linear	Quadratic	Linear	Linear	Linear
β_0	762.48	51.88	7.51	7.29	75.30	75.50
β_1	415.43	25.62	3.09	-0.46	0.26	0.19
β_2	370.04	0.00	0.74	-3.54	-7.10	-7.95
β_3	0.00	0.00	-0.71	-0.82	-1.89	-3.01
$\beta_1\beta_2$	192.82	0.00	0.00	0.00	0.00	0.00
$\beta_1\beta_3$	0.00	0.00	0.00	0.00	0.00	0.00
$\beta_2\beta_3$	0.00	0.00	0.00	0.00	0.00	0.00
β_{11}	0.0091	0.00	0.00	0.00	0.00	0.00
β_{22}	125.68	0.00	0.00	0.00	0.00	0.00
β_{33}	-2.83	0.00	0.00	0.00	0.00	0.00

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 + \beta_{33} {X_3}^2 + \beta_1\beta_2 X_1 X_2 + \beta_1\beta_3 X_1 X_3 + \beta_2\beta_3 X_2 X_3$$

Note: Y_1 = total drying time (min), Y_2 = total heating time during intermittent (min), Y_3 = total colour change, Y_4 = nitrite content change (ppm), Y_5 = sialic acid content (mg/g) and Y_6 = antioxidant content (mg/g).

Figures 4.21-4.26 show the three-dimension response surfaces and contour plots of the predictive polynomial models. The response surfaces and contour plots of the response variables are in functions of intermittent duration (X_1) and intermittent ratio (X_2) by setting the intermittent cycle at constant value (X_3 = 2). Figure 4.20 shows the quadratic relationship between total drying time and drying variables. It can be seen that shorter total drying time can be obtained when a short intermittent duration is coupled with high intermittent ratio (longer heating time during an intermittent cycle).

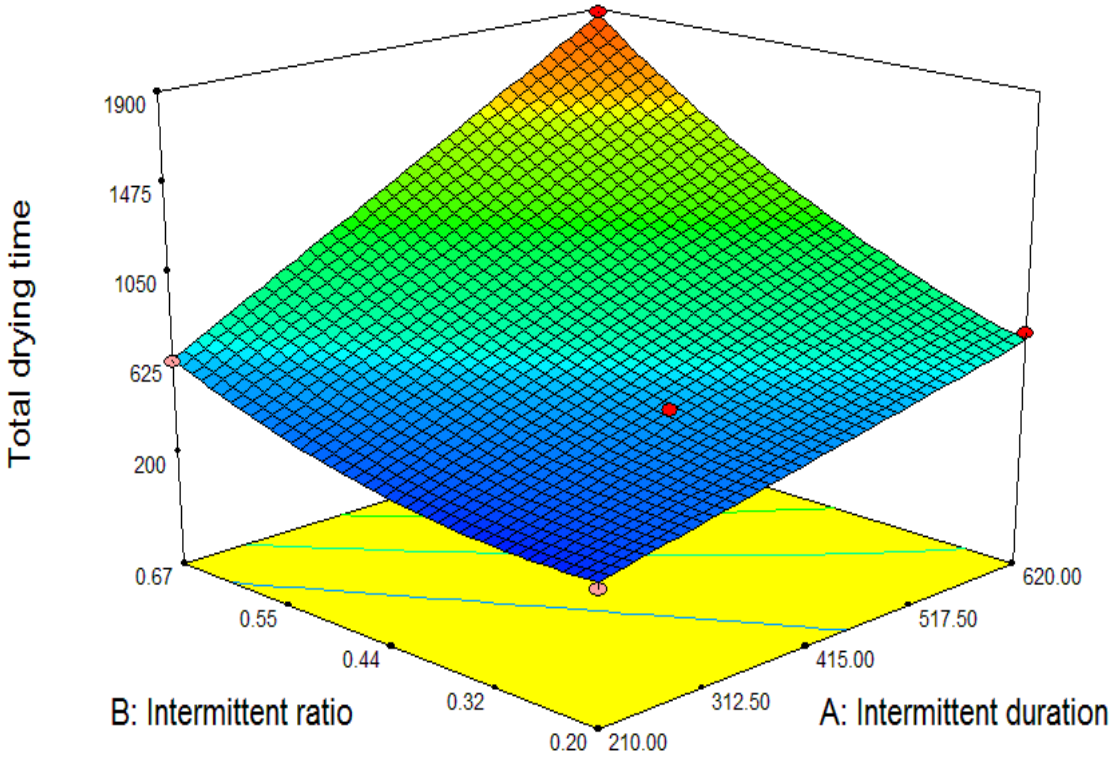


Figure 4.21a Three-dimension response surface of total drying time as a function of intermittent duration and intermittent ratio.

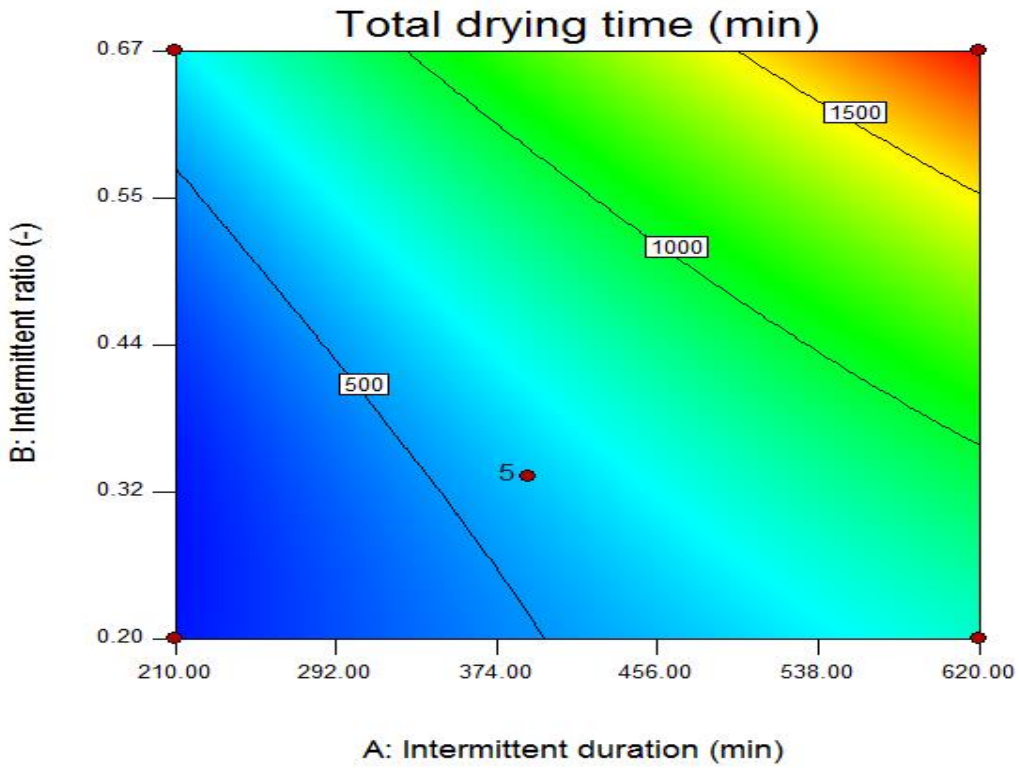


Figure 4.21b Contour plot of total drying time as a function of intermittent duration and intermittent ratio.

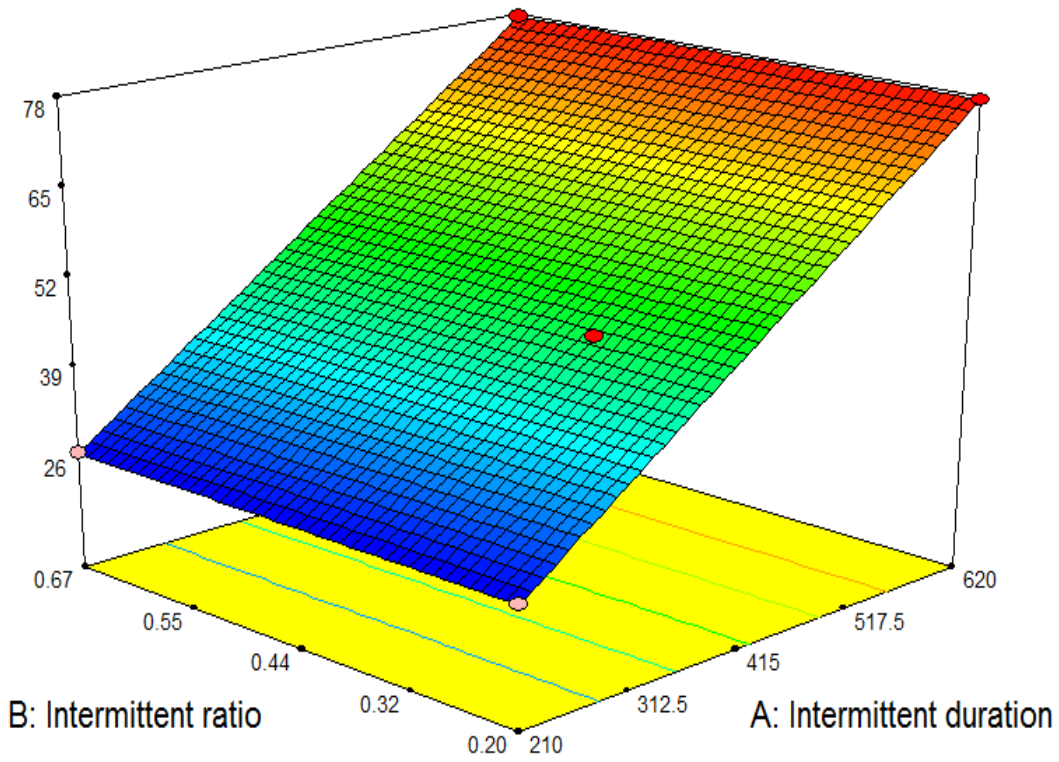


Figure 4.22a Three-dimension response surface of total heating time during intermittent as a function of intermittent duration and intermittent ratio.

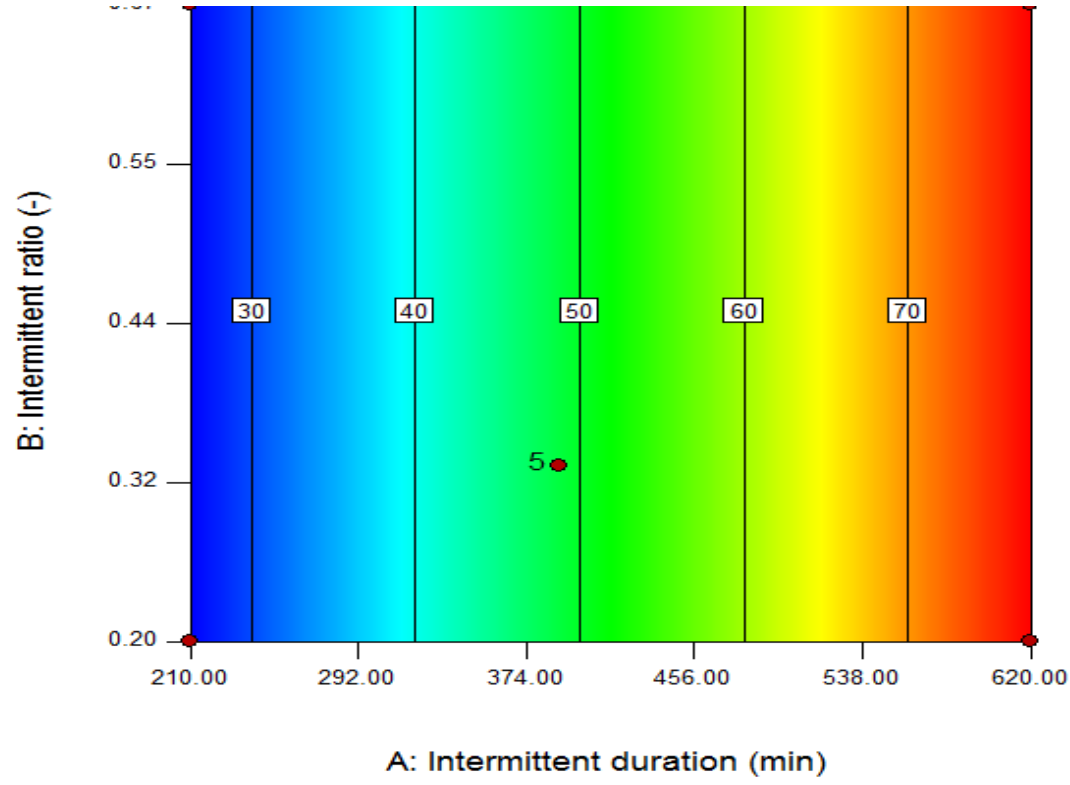


Figure 4.22b Contour plot of total heating time during intermittent as a function of intermittent duration and intermittent ratio.

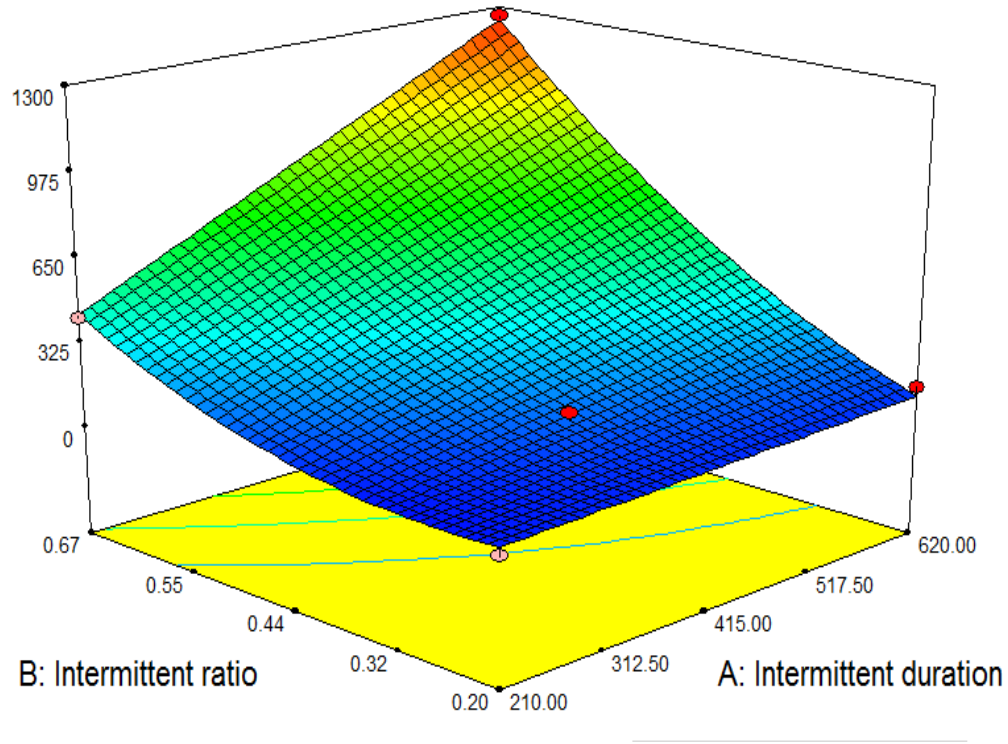


Figure 4.23a Three-dimension response surface of total colour change after intermittent as a function of intermittent duration and intermittent ratio.

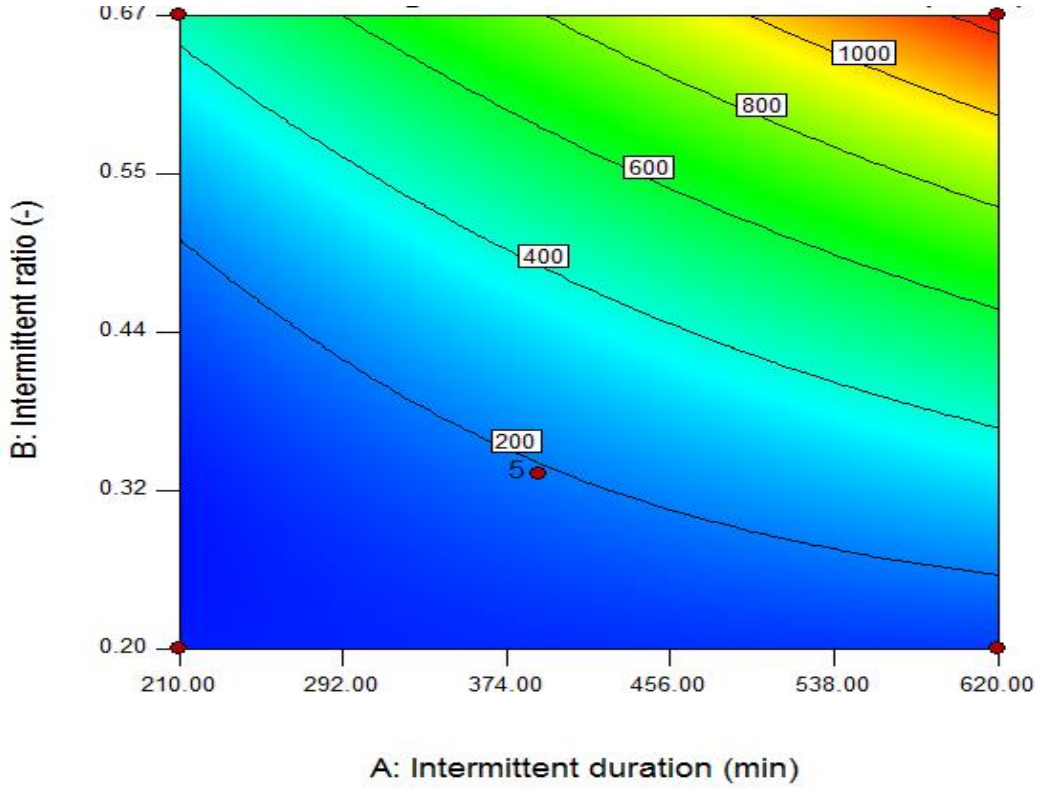


Figure 4.23b Contour plot of total colour change after intermittent as a function of intermittent duration and intermittent ratio.

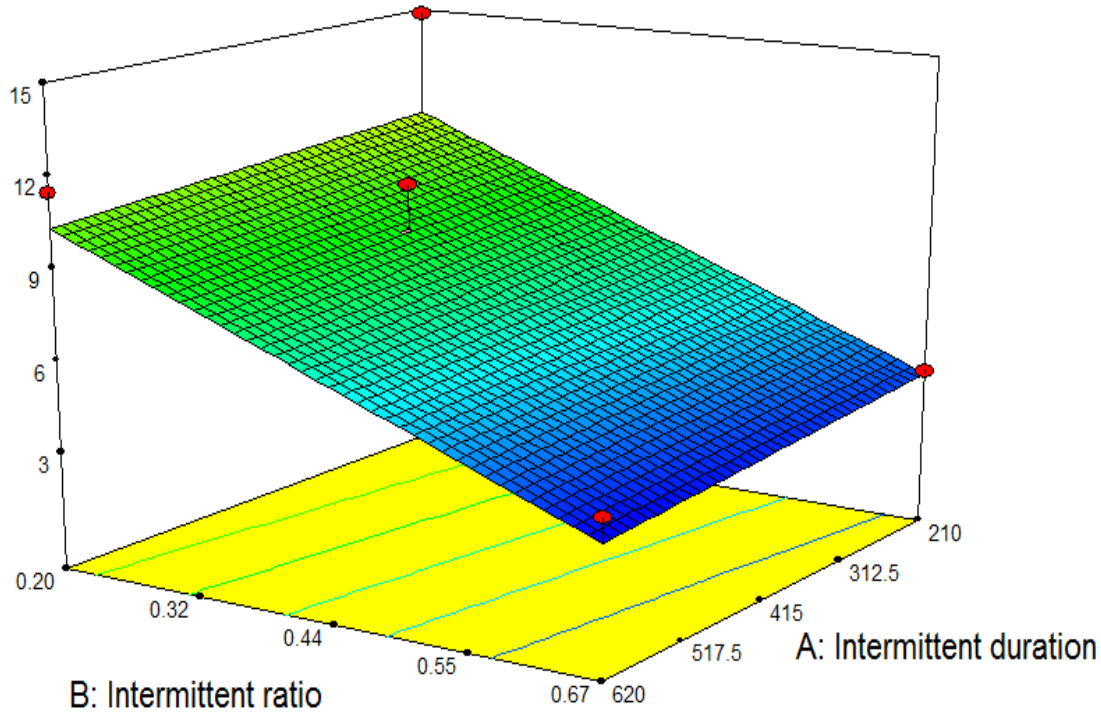


Figure 4.24a Three-dimension response surface of nitrite content change as a function of intermittent duration and intermittent ratio.

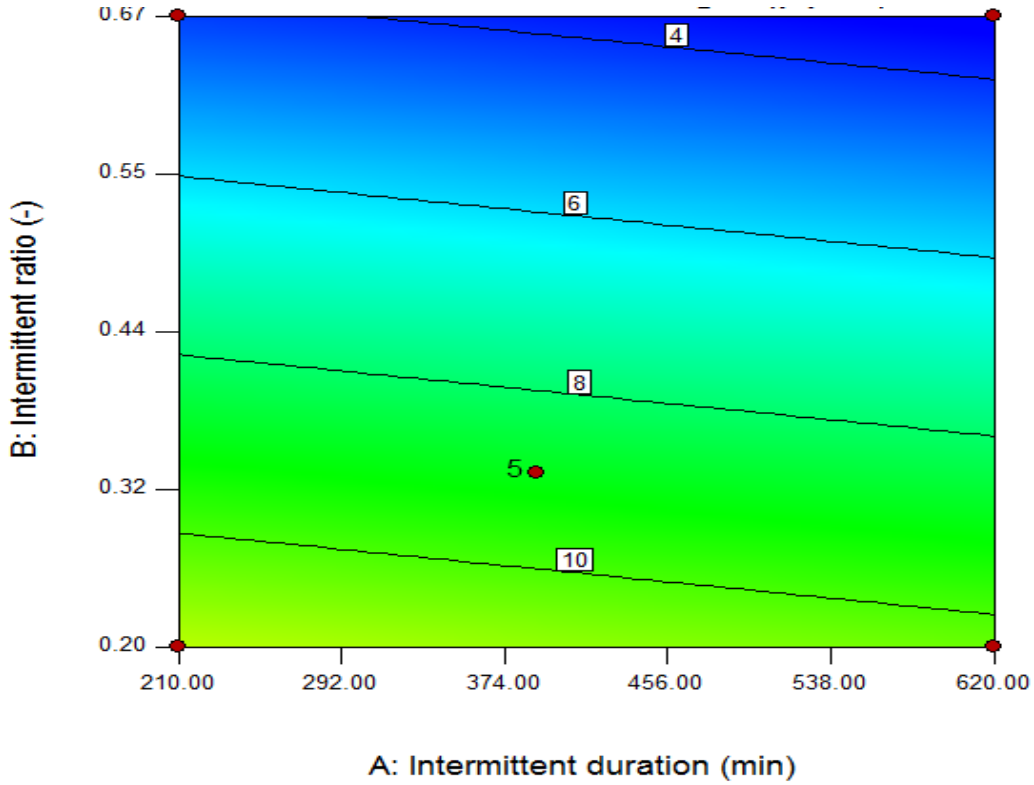


Figure 4.24b Contour plot of nitrite content change as a function of intermittent duration and intermittent ratio.

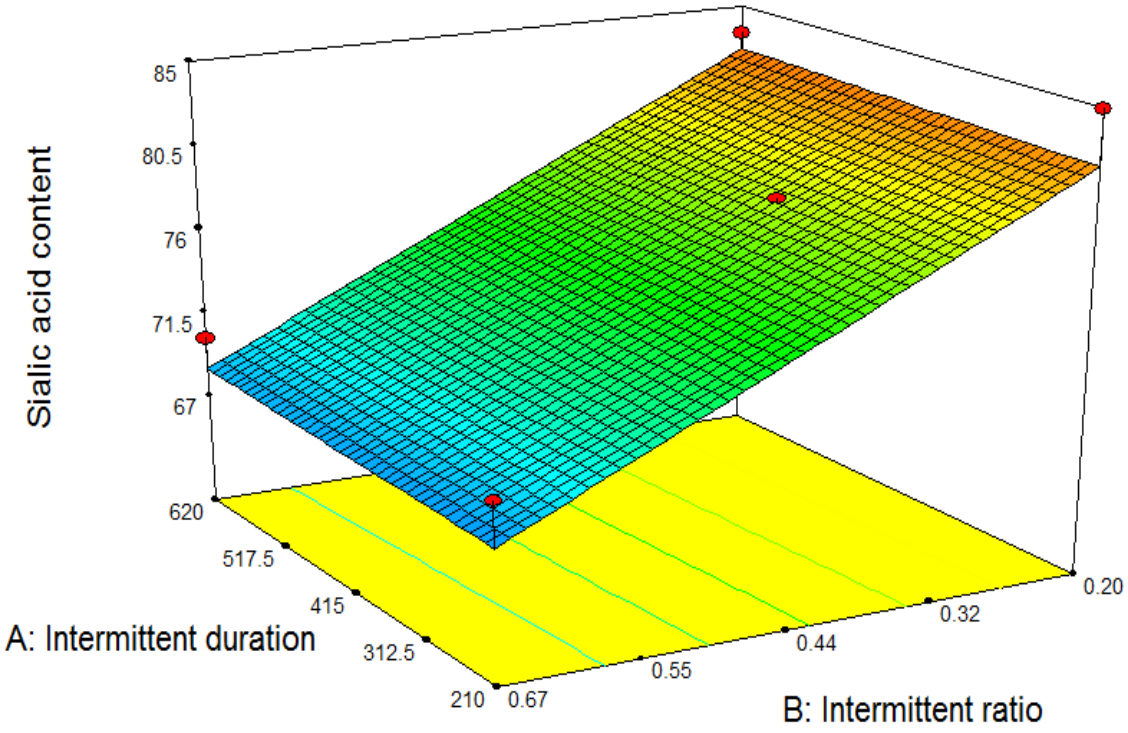


Figure 4.25a Three-dimension response surface of sialic acid content as a function of intermittent duration and intermittent ratio.

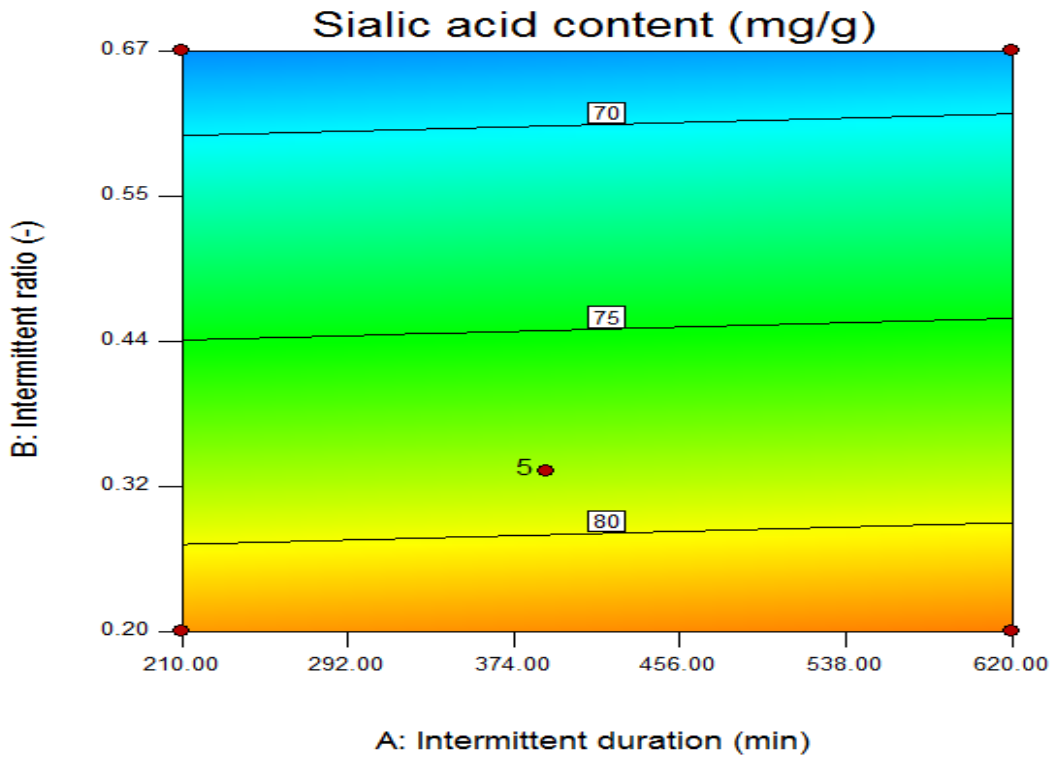


Figure 4.25b Contour plot of sialic acid content as a function of intermittent duration and intermittent ratio.

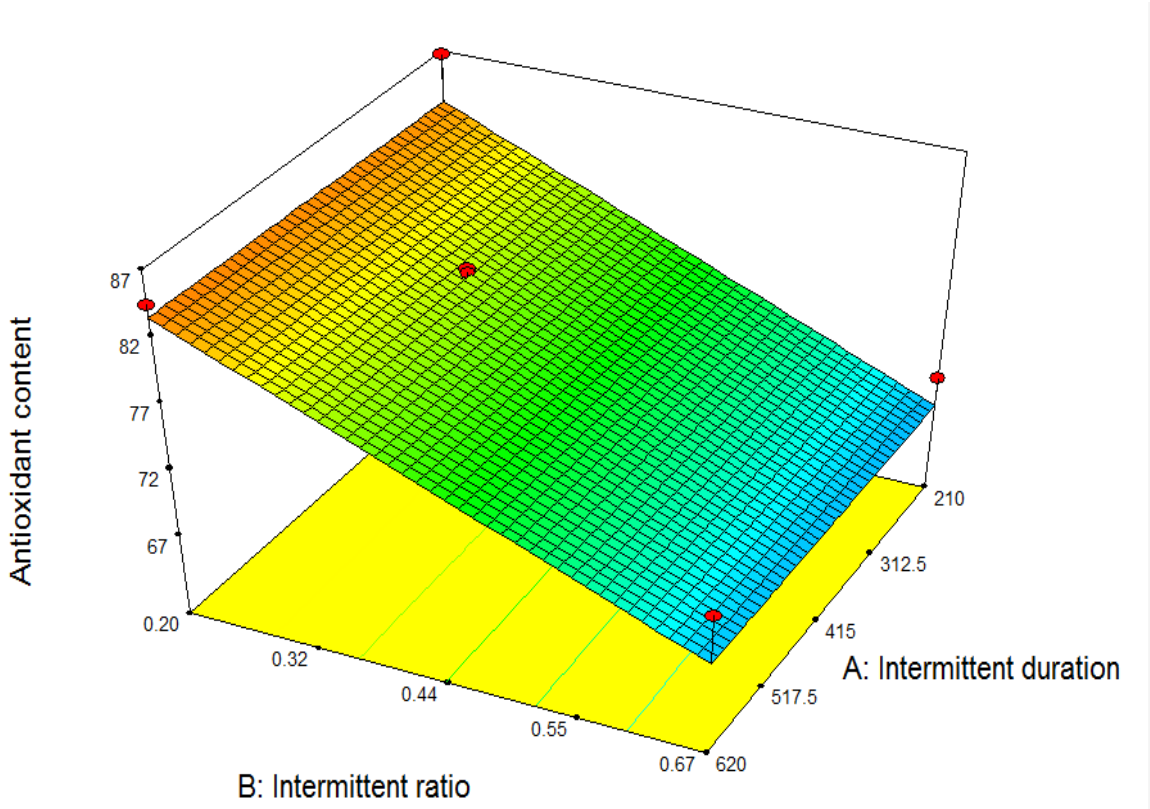


Figure 4.26a Three-dimension response surface of antioxidant content as a function of intermittent duration and intermittent ratio.

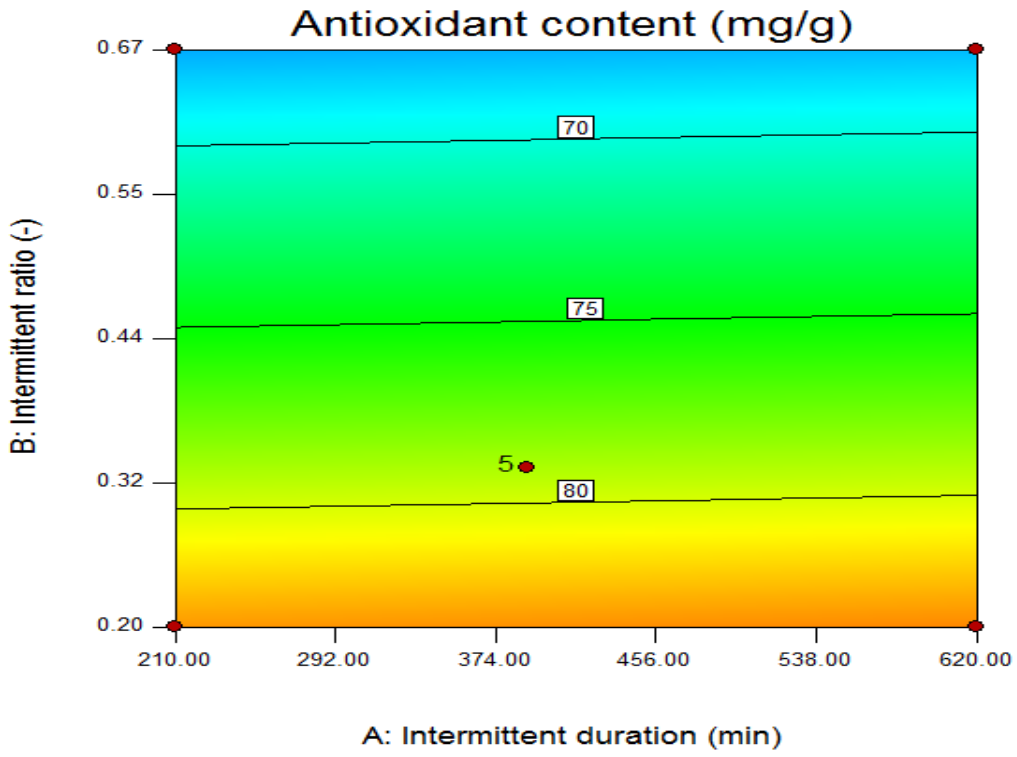


Figure 4.26b Contour plot of antioxidant content as a function of intermittent duration and intermittent ratio.

The shorter total heating time during intermittent period is achievable as shown in Figure 4.22 when a short intermittent duration and low intermittent ratio is employed. On the other hand, long intermittent duration and high intermittent ratio have to be applied in order to obtain shorter total heating time after intermittent period (Figure 4.23). Hence, it can be concluded that shorter intermittent period and longer continuous heating time could shorten the total drying time. However, in order to obtain optimum product quality, an optimum combination has to be determined. Meanwhile, Figure 4.24 shows that the nitrite content of samples would increase with longer intermittent duration and higher intermittent ratio (shorter drying time). This is probably due to the multiplication of nitrobacteria at the longer tempering period that may lead to the increased nitrite content in samples.

In contrary, it is observed that retention of sialic acid and antioxidant are higher in Figures 4.25 and 4.26 when short intermittent duration but high intermittent ratio (shorter total drying time) is applied. The observation shows that reduction of sialic acid and antioxidant contents is due to thermal degradation. With shorter heating time, the product experiences lesser heat damage; hence, the heat sensitive bioactive ingredients such as sialic acid and antioxidant in EBN could be retained. As for the sialic acid and antioxidant contents, the quadratic response surface demonstrates that there is an optimum retention at intermittent ratio between 0.40-0.44. By employing the optimal intermittent drying parameters, the transport properties (moisture content and water activity), physiochemical properties (colour degradation, rehydration capacity and shrinkage) and also the biochemical properties (nitrite content, retention of sialic acid content and retention of total antioxidant capacity) of the dried EBN samples are studied.

Table 4.17 The obtained product qualities by performed best drying method with optimal drying parameters.

Optimal drying parameters	
Drying method	Intermittent IR-UVC assisted drying at 40°C (IR-UVC40)
Intermittent duration	415-min
Intermittent ratio	0.40
Intermittent cycle	3
Product Qualities	
Transport Properties	
Equilibrium moisture content (EMC)	10.38% dry basis
Water activity, a_w	0.42
Effective moisture diffusivity, D_{eff}	4.61×10^{-10} m^2s^{-1}
Effective thermal diffusivity, α_{eff}	4.65×10^{-10} m^2s^{-1}
Physiochemical Properties	
Total colour change, ΔE	1.87 ± 0.10
Dry basis holding capacity (DHC)	0.99 ± 0.10
Water Absorption Capacity (WAC)	0.97 ± 0.08
Rehydration Ability (RA)	0.96 ± 0.05
Shrinkage	0.91 ± 0.03
Biochemical Properties	
Nitrite content	2.08 ± 0.17 ppm
Retention of sialic acid	92.84 ± 0.53 mg/g
Retention of total antioxidant capacity	90.20 ± 0.11 mg/g

Mean values ± standard deviation (n = 3 replications) within the same column with the same letter are not significantly different (p>0.05).

The results shown in Table 4.17 were proved that the EBN samples dried by intermittent IR-UVC drying at 40°C, 16.5% RH and α= 0.40 had the best product qualities. The total colour change of EBN dried sample was not significantly different (p>0.05) and the least changes as compared to fresh sample; also, the least shrinkage and least increment of nitrite content were observed in the dried sample. The sialic acid and total antioxidant capacity also were well preserved in the EBN dried sample, where the retention percentage of sialic acid and total antioxidant capacity were recorded at 92% and 90%, respectively. Therefore, in the present study, the optimal intermittent drying parameters are determined at intermittent duration of 415-min, at intermittent ratio of 0.40 and intermittent cycle of 3, in order to obtain the best product quality using the shortest drying time.

CHAPTER 5 CONCLUSION AND FUTURE WORKS

5.1 Conclusion

The present study represents the first investigation on drying of edible bird's nest (EBN) and its bioactive ingredients such as sialic acid and antioxidants through intermittent IR-UVC assisted drying which has not been reported elsewhere. It can be seen that hot air drying at 80°C, 8.3% RH and α=1.00 gave the highest drying rate and followed by IR-UVC assisted drying at 40°C, 16.5% RH and α =0.33. The effective moisture diffusivity, D_{eff} values increased with drying temperature in both continuous IR-UVC assisted drying at 40°C and hot air drying at 80°C with values ranging from 4.58 x 10^{-10} m^2s^{-1} to 1.50 x 10^{-9} m^2s^{-1}. The results also indicate that effective thermal diffusivity would increase with increasing temperature. This trend can be seen clearly from the hot air dried samples at 80°C and IR-UVC assisted dried samples at 40°C, where effective diffusivity increases from 5.00 x 10^{-10} m^2/s to 6.50 x 10^{-10} m^2/s when drying temperature increases from 40°C to 80°C.

Besides, it can be seen that total colour change (ΔE) of dried samples from intermittent IR-UVC drying (IR-UVC25 and IR-UVC40) are significantly ($p<0.05$) lower than that in continuous mode. On the other hand, the colour of dried samples obtained by intermittent IR-UVC assisted drying (IR-UVC25 and IR-UVC40) at α = 0.20, 0.33 and 0.67, respectively, did not change significantly ($p>0.05$) and remained almost the same as the fresh EBN even after 1-month storage. Moreover, the rehydration ability of intermittent IR-UVC assisted dried samples is significantly ($p<0.05$) higher than continuous IR-UVC assisted dried samples. In terms of shrinkage, continuous IR-UVC assisted dried samples shrunk significantly ($p<0.05$) when compared with intermittent IR-UVC dried samples.

Interestingly, the nitrite content of fan assisted dried samples is significantly *(p<0.05)* higher than IR-UVC assisted dried samples. The nitrite content of IR-UVC assisted dried samples reduced significantly *(p<0.05)* as compared to IR or UVC assisted dried samples. Also, the sialic acid content in sample dried by intermittent IR-UVC assisted drying is significantly (*p<0.05*) higher than sample dried by continuous IR-UVC assisted drying, fan assisted drying and hot air drying. The retention of sialic acid by intermittent IR-UVC assisted drying at 25°C and 40°C were up to 83.9% and 78.7%, respectively; while the retention of sialic acid by continuous hot air drying at 80°C is only 42.5% if compared with the fresh sample. On the other hand, the highest antioxidant content preserved in EBN samples is from intermittent IR-UVC assisted drying at 25°C (85.61± 0.09 mg TEAC/g dry weight), followed by intermittent IR-UVC assisted drying at 40°C (81.18 ± 0.07 mg TEAC/g dry weight); while the lowest antioxidant activity is from continuous hot air-dried EBN sample at 80°C (34.28 ± 0.14 mg TEAC/g dry weight). The results suggest that drying temperature in IR-UVC assisted dryer at 40°C is sufficiently low to minimize thermal destruction of sialic acid and total antioxidant activity in EBN.

In this study, an optimization on three drying variables, such as intermittent duration (X_1), intermittent ratio (X_2) and intermittent cycle (X_3) was performed by response surface methodology with the aims to determine the best combination of the drying variables, in order to avoid excessive long drying period and also to improve product quality and storage stability. The optimization studies showed that EBN samples dried by intermittent IR-UVC drying at 40°C, 16.5% RH and α= 0.40 had the best product quality. The total colour change of dried EBN sample was not significantly different (p>0.05) and showed the least changes as compared to fresh sample; also, the least shrinkage and least increment of nitrite content were observed in similar sample. The sialic acid and total antioxidant capacity were also well preserved in the EBN dried sample where the retention of

sialic acid and total antioxidant capacity were recorded at 92% and 90%, respectively. Therefore, in the present study, the optimal intermittent drying parameters are determined at intermittent duration of 415-min, intermittent ratio of 0.40 and intermittent cycle of 3.

5.2 Future Works

The present study has revealed the potential of intermittent IR-UVC assisted drying as an alternative method for drying of edible bird's nest (EBN) and preservation of its important bioactive ingredients. The potential of IR-UVC assisted drying should be further explored to provide a complete drying technology that will contribute to the knowledge enhancement in EBN drying.

The following future works are therefore recommended:

i. Performance and cost analyses of the IR-UVC assisted dryer based on the drying conditions from this research.
ii. Determination of the thermophysical properties of EBN by experimental method and correlate with moisture content to improve heat and mass transfer modelling.
iii. Incorporation of heat pump to the conventional low temperature dryer currently used in this study for pilot scale trials.
iv. Investigation on the degradation kinetics of sialic acid and antioxidant contents in EBN under heat pump assisted drying and compare with intermittent IR-UVC assisted drying.

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APPENDIX A

Figure A1 Actual diagram of IR-UVC dryer.

Figure A2 Actual diagram of inner compartment of dryer.

APPENDIX B

Figure B1 Actual diagram of ultraviolet C disinfection storage cabinet.

Figure B2 Actual diagram of inner compartment of the storage cabinet.

APPENDIX C

Table C1 Summary of standard for raw clean edible bird's nest under Health Food China Standard.

Official Control	Purpose	Type of Food	Parameter of Analysis	Tolerance Level	China Standard
Microbiological Criteria	Microbiology criteria for raw clean edible bird's nest	Raw clean edible bird's nest	1) Total Plate Count (TPC)	≤30,000 cfu/g when protein ≥4%, ≤1,000 cfu/g when protein <4%	GB 16740-1997 Health Food China Standard
			2) Coliform	≤90 MPN/100g when protein ≥4%, ≤40 MPN/100g when protein <4%	
			3) E.coli	Not Detected	
			4) Salmonella spp.	Not Detected	
			5) Staphylococcus Aureus	Not Detected	
			6) Yeast	≤ 25 cfu/g	
			7) Mould	≤ 25 cfu/g	
Chemical	Maximum level for nitrite in raw edible bird's nest	Raw clean edible bird's nest	Nitrite	30 mg/kg (ppm)	
Heavy Metal	Maximum level of heavy metal	Raw clean edible bird's nest	1) Arsenic (As)	≤ 1.0 mg/kg (ppm)	
			2) Plumbum (Pb)	≤ 0.5 mg/kg (ppm)	
			3) Mercury (Hg)	≤ 0.05 mg/kg (ppm)	
			4) cadmium (Cd)	≤ 1.0 mg/kg (ppm)	
Minerals	Minerals in raw clean edible bird's nest	Raw clean edible bird's nest	1) Copper (Cu)	2.0 mg/kg (ppm)	
			2) Iron (Fe)	0.3 mg/kg (ppm)	

APPENDIX D Calibration Curve of Sialic Acid and Monoses

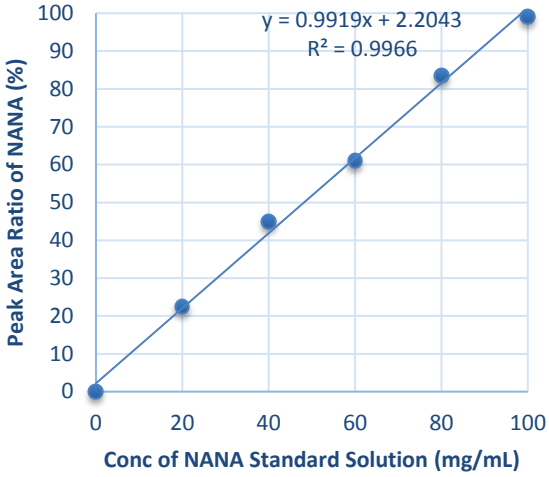

Figure D1 Calibration curve of NANA.

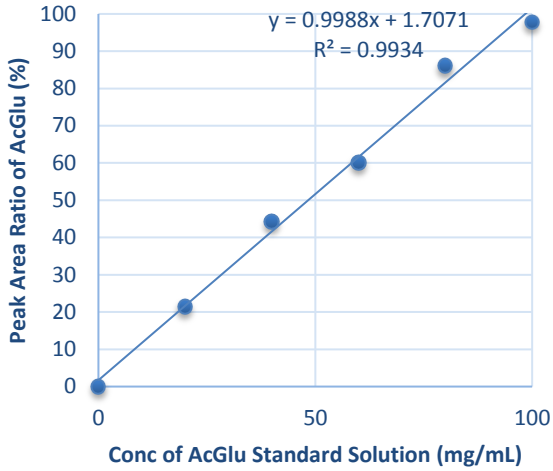

Figure D2 Calibration curve of AcGlu.

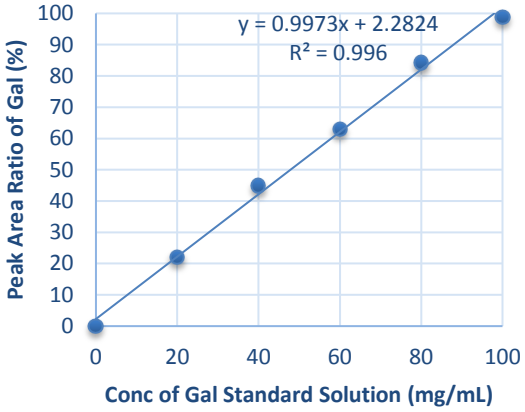

Figure D3 Calibration curve of Gal.

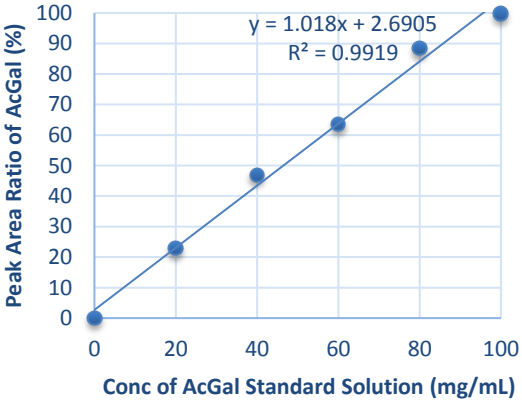

Figure D4 Calibration curve of AcGal.

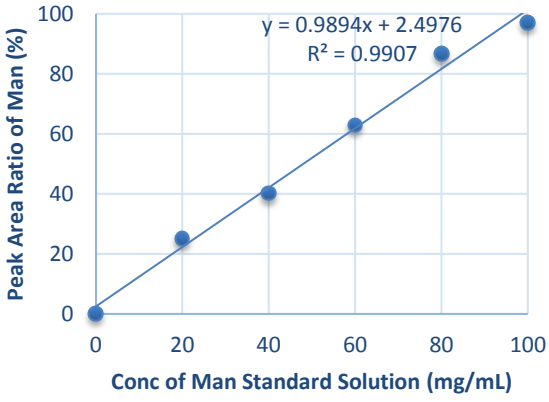

Figure D5 Calibration curve of Man.

APPENDIX E Validation of Analytic Method for Sialic Acid

Table E1 Calibration curves, linearity range, limit of detection and limit of quantification of monoses as detected by HPLC.

Analyte	Linear Equation	R^2	Linearity Range	LOD	LOQ
N-Acetyl-neuraminic acid (NANA)	y = 0.9919 x + 2.2043	0.9966	0.02 –100	0.01	0.02
N-Acetyl-$_D$-glucosamine (AcGlu)	y = 0.9988 x + 1.7071	0.9934	0.02 –100	0.01	0.02
D-Galactose (Gal)	y = 0.9973 x + 2.2824	0.9960	0.02 –100	0.01	0.02
N-Acetyl-$_D$-galactosamine (AcGal)	y = 1.018 x + 2.6905	0.9919	0.02 –100	0.01	0.02
$_D$-Mannose (Man)	y = 0.9894 x + 2.4976	0.9907	0.02 - 100	0.01	0.02

Table E2 Intra-day precisions of five standard monoses as detected by HPLC.

Analyte	Intra-day precision (RSD, %)				
	20 mg/mL	40 mg/mL	60 mg/mL	80 mg/mL	100 mg/mL
N-Acetyl-neuraminic acid (NANA)	3.3	3.8	4.4	5.1	3.7
N-Acetyl-$_D$-glucosamine (AcGlu)	3.7	3.5	4.3	5.0	4.5
D-Galactose (Gal)	4.1	5.4	5.7	5.3	5.0
N-Acetyl-$_D$-galactosamine (AcGal)	8.1	8.8	8.6	8.2	9.6
$_D$-Mannose (Man)	9.2	10.6	9.5	9.1	9.4

Table E3 Recovery % of five standard monoses as detected by HPLC.

Analyte	Recovery (%)				
	20 mg/mL	40 mg/mL	60 mg/mL	80 mg/mL	100 mg/mL
N-Acetyl-neuraminic acid (NANA)	93.5	91.2	90.6	94.5	95.5
N-Acetyl-$_D$-glucosamine (AcGlu)	80.5	87.9	88.2	90.1	81.4
D-Galactose (Gal)	71.4	78.5	80.3	77.7	76.9
N-Acetyl-$_D$-galactosamine (AcGal)	72.6	83.7	80.5	73.0	75.1
$_D$-Mannose (Man)	78.9	84.1	86.6	80.9	82.2

APPENDIX F Calibration Curve of Antioxidant

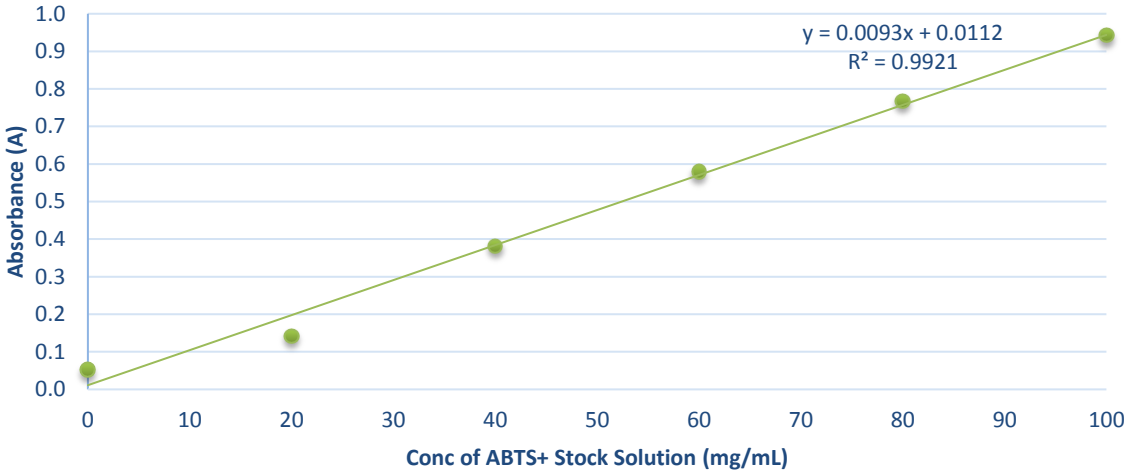

Figure F1 Calibration curve of standard ABTS⁺ stock solution.

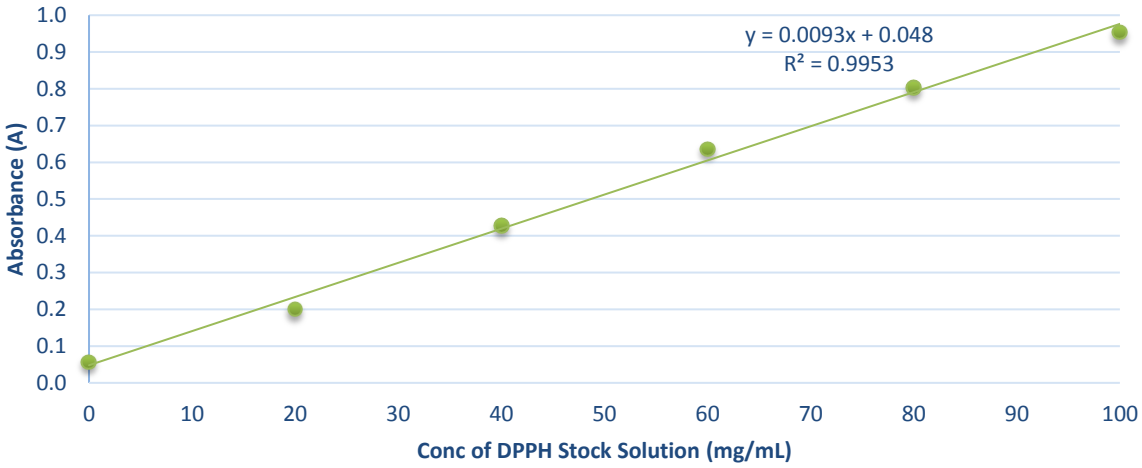

Figure F2 Calibration curve of standard DPPH stock solution.

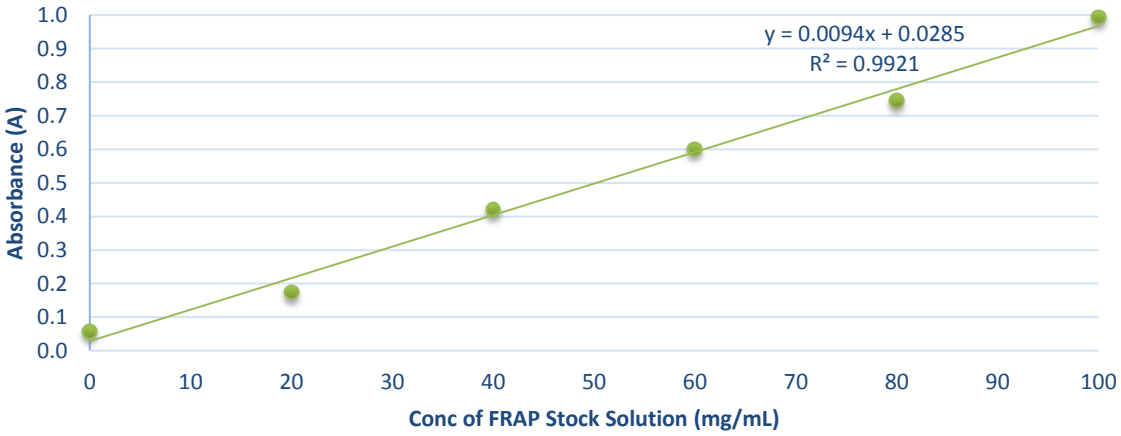

Figure F3 Calibration curve of standard FRAP stock solution.

APPENDIX G Validation of Analytic Method for Antioxidant

Table G1 Calibration curves, linearity range, limit of detection and limit of quantification of three assays (ABTS, DPPH and FRAP).

Assay	Linear Equation	R^2	Linearity Range	LOD	LOQ
ABTS	y = 0.0093 x + 0.0112	0.9921	0.02 – 100	0.01	0.02
DPPH	y = 0.0093 x + 0.0480	0.9953	0.02 – 100	0.01	0.02
FRAP	y = 0.0094 x + 0.0285	0.9921	0.02 – 100	0.01	0.02

Table G2 Intra-day precisions of three stock solutions (ABTS, DPPH and FRAP) at different concentration levels.

Assay	Intra-day precision (RSD, %)				
	20 mg/mL	40 mg/mL	60 mg/mL	80 mg/mL	100 mg/mL
ABTS	2.8	3.3	3.5	4.0	3.7
DPPH	3.3	3.0	3.4	5.0	4.2
FRAP	4.4	3.7	4.6	3.5	4.0

Table G3 Inter-day precisions of three stock solutions (ABTS, DPPH and FRAP) at five different concentration levels.

Assay	Inter-day precision (RSD, %)				
	20 mg/mL	40 mg/mL	60 mg/mL	80 mg/mL	100 mg/mL
ABTS	3.5	3.0	3.0	3.6	3.7
DPPH	3.3	2.9	3.0	3.2	2.8
FRAP	4.7	3.1	4.2	3.5	4.0

Table G4 Recovery percentage of three stock solutions (ABTS, DPPH and FRAP) at five different concentration levels.

Assay	Recovery (%)				
	20 mg/mL	40 mg/mL	60 mg/mL	80 mg/mL	100 mg/mL
ABTS	95.2	93.6	90.1	87.9	90.6
DPPH	90.3	92.2	86.6	85.2	89.4
FRAP	89.1	89.9	92.4	83.0	90.3