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A novel process to separate the eggshell membranes and eggshells via flash evaporation

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Abstract
Eggshell membrane is a kind of cheap, abundant resources with great potential utilization value. A variety of active ingredients in it can be used as raw materials for medicine, light industry and other industries. At present, soluble protein, collagen, keratin, sialic acid, hyaluronic acid and antimicrobial protein have been successfully extracted from eggshell membrane and exploited in various fields. This study aimed to separate the eggshell membrane for fully exploiting the potential utilization value of the eggshell, and reduce the amount of waste discharged, which is conducive to protecting the ecological environment on which we depend. In order to reduce energy consumption of eggshell membrane separation, separate eggshell membranes from eggshells by flash evaporation was investigated. The flash separation conditions were optimized considering three independent variables at three different levels by response surface methodology. The results showed that the processing parameters of separating eggshell membranes and eggshells with flash evaporation were 1.0 Mpa pressure, 45% moisture content, and 5 mm eggshells' particle size. Under these conditions, the separation rate was 69.16%. Therefore, flash separation can be a promising approach for separating eggshells and eggshell membranes.

Keywords: eggshell membrane; eggshells; flash evaporation; flash separation.

Practical Application: In this study, separate the eggshell membranes and eggshells with flash evaporation was creatively proposed and analyzed the factors affecting the separation rate. Flash separation is an efficient and eco-friendly method, which is of great significance to eggshell membrane separation industry.

1 Introduction

The global demand and production of eggs is ever-increasing. In 2018, global production of eggs was 76.7 million tons, an increase of 14.95% over the past 10 years (Shahbandeh, 2020). China produced 26.9 million tons in 2018 (Food and Agriculture Organization, 2020). In the same year, the United States became the second largest producer of eggs with a total annual production of 6.46 million tons, followed by India (5.23 million tons), Mexico (2.87 million tons), Brazil (2.66 million tons) and Japan (2.62 million tons) (Food and Agriculture Organization, 2020). The increase in egg production worldwide is a result of its high global consumption and egg consumption is estimated to rise by 50% up to 2035, mainly because of its lower price compared to other protein sources and its perceived health benefits (Sass et al., 2021).

Therefore, understanding consumer behavior to improve production, technology and marketing strategies in this field can avoid unnecessary waste (Sass et al., 2020). Due to their high perishability over the storage period, the treatment (thermal or coatings) is an excellent way to promote egg conservation (Oliveira et al., 2020). Eggs have other applications beyond human consumption. For example, foam mat drying with egg albumen foam was applied in drying ripe bananas into flour (Noordia et al., 2020).

Eggshell constitutes around 10% of a hen's egg by weight (Laca et al., 2017). A large number of eggs are produced each year, of which a large proportion (30%) are processed in the food industry, resulting in a large increase in eggshell waste (Ahmed et al., 2019). The rotting of eggshells is a source of environmental pollution, so disposing of egg waste is not a pleasant task (Tsai et al., 2008). The massive increase in egg production has resulted in increased generation of eggshell waste. Tons of discarded eggshells contribute to food waste which causes significant damage to the environment by increasing global carbon footprint (3.3 Gtons of CO2 equivalent in 2007) when buried (Food and Agriculture Organization, 2013), which is one of the major greenhouse gases contributing to the global warming (Waheed et al., 2019).

Eggshell is an important part of the egg. From the outside to the inside, it is mainly divided into two parts: eggshell and eggshell membrane. The main component of eggshell is CaCO3, which is a natural high-quality environmental calcium source. After treatment, it can be transformed into calcium lactate, calcium gluconate, calcium acetate and other biological organic calcium products, which can be used as medical calcium fortifiers and food additives. The eggshell membrane mainly consists of individual fibers and can be divided into 3 parts: the outer eggshell membrane, the inner eggshell membrane, and the limiting membrane (Baláž, 2014). The membrane contains soluble polymer compounds such as N-acetylglucosamine galactose, glucuronic acid, hyaluronic acid, chondroitin sulfate, amino acids, etc, which can be widely used in medicine and skin care products. Some studies have shown that membrane can be used

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as a biological adsorbent (Tsai et al., 2006), enzyme biosensor immobilized membrane (Zhang et al., 2006) and new drug controlled release and medical dressing materials (Ino et al., 2006). Eggshells strengthens bone tissues, removes radioactive elements and is also incorporated in various supplements by pharmaceutical companies' (Wellman-Labadie et al., 2007). Furthermore, in rat studies, it was discovered that calcium obtained from eggshells powder has a high bioavailability as compared to the commercially available calcium carbonate (Świątkiewicz et al., 2015). Despite the importance of these applications, eggshells are still underutilized and further research is needed to increase their value.

Separating eggshell membranes from eggshells is an urgent project, which is still in the stage of research and exploration, has not been applied in the industry so far. There are three methods through searching the references of papers and patents for separating eggshell membranes from eggshells, physical method, chemical method and enzymatic method (Li, 2020). The separation of eggshells and eggshell membranes by physical method mainly rely on grinding or cracking (Wang et al., 2012) which could cause dust pollution to the air. The chemical method mainly soaked eggshells in acid or alkali solution (Su et al., 2016) which would pollute the ecological environment, affects the physical and chemical characteristics of eggshell membrane, and restricts the application of eggshell membrane. The long chemical reaction time results the long separation period. The enzymatic method hydrolyzed eggshell membrane with protease (Zhao et al., 2008) which required harsh reaction conditions, and increased the production cost of eggshell membrane separation with expensive protease. This paper used flash separation which belongs to physical method. Flash separation is widely used in modern industry, including seawater desalination, waste heat recovery, food and drug processing and drying, etc. Since the 1970s, scholars have begun to study the flash process in detail, and used different working medium, testing equipment and observation angle to analyze the flash process comprehensively, and achieved great success.

From the foregoing discussion, it is evident that previous studies of separating eggshell and eggshell membrane have their flaws. The interest of this study was to use a new method to separate eggshell membranes from eggshells which is flash evaporation. Compared with other physical methods, flash separation has the advantages of simple structure equipment and low energy consumption. The experimental conditions of flash evaporation were easy to achieve. Flash separation was an eco-friendly method which could not cause dust pollution and chemical contamination. In this experiment, the separation rate was measured on the condition of 30 ~ 50% moisture content and 0.8 ~ 1.0 MPa pressure.

2 Material and methods

2.1 Composition and characterization of eggshell

The chemical composition of eggshell and eggshell membrane

The eggshell is mainly composed of inorganic substances, which account for 94-97% of the whole eggshell. The main inorganic substance in eggshell is $CaCO_3$ with the content up to 93%, in which calcium content is more than 36%. The organic content of eggshell is only 3% ~ 6%, which is mainly protein (Zhang & Zhao, 2009). Eggshell matrix proteins were analyzed by immunofluorescence and colloidal gold immunocytochemistry techniques. The identified eggshell matrix proteins can be divided into general proteins, egg white proteins and eggshell formation specific proteins. Common proteins include osteopontin and agglutinin; egg white protein is mainly composed of ovalbumin, lysozyme and ovaltransferin, of which the contents of lysozyme and ovaltransferin are higher than ovalbumin in eggshell membrane (Yu et al., 2008). Carbohydrate content is minimal, but it is rich in nutrients, main polysaccharides, galactosamine, hexosylamine, glucosamine, mannose, galactose, fructose and so on.

The main component of the eggshell membrane is protein, which exists in the form of glycoproteins, similar to the composition of cell membrane proteins. The eggshell membrane contains 70% organic, 10% inorganic and 20% water (Craig & Reg, 2002). Organic matter contains about 90% protein, 3% liposomes and 2% sugars, the proteins are keratin, collagen (mostly I, V, X type), complex proteins, etc, rich in hydroxyproline, hydroxy sine and cystine. The eggshell membrane also contains a lot of soluble polymer compounds, such as N-acetylglucosamine galactose, glucuronic acid, hyaluronic acid, chondroitin sulfate, etc. The minerals are calcium, magnesium, strontium and almost free of lead, aluminum, cadmium, mercury, iodine. In addition, the eggshell membrane contains sticky carbohydrates (Wang et al., 2010).

Structure and characterization of eggshell and eggshell membrane

The eggshell thickness is about 0.3 mm, accounting for about 11% of the total egg weight, and the density is 2300 kg/m^3. The structure of eggshell from the outside to the inside is successively: inner and outer eggshell membrane layer, papillary layer, gate layer, vertical crystalline layer, epidermis layer and fibrous membrane layer (Zhang et al., 2005). Some scholars also divide the eggshell into three layers: the inner layer of the papillary layer, the middle sponge layer and the outer cuticle layer, the middle sponge layer is the barrier layer and the vertical crystalline layer (Bin et al., 2006). The epidermis is the outermost surrounding part of the eggshell, which is mainly composed of substances containing more calcium salts, generally proteins, carbohydrates and fats. Its structure is basically continuous, which plays the role of waterproof layer, and is also the first barrier to prevent microorganisms from entering. The gate layer is the part that radiates from the inner protruding layer to the epidermis layer, and is the main component of the eggshell with a lamellar crystal structure. Papillary layer to layer of eggshell, eggshell crystal growth from this, generally for papillary, contained more milky cone, cone body hold air holes between the two, namely porosity, grid layer and the papillary layer is mainly composed of protein as matrix of composite materials, including 95% of the calcium carbonate, 3.3% protein and 1.6% water, grid layer is relatively dense and hard. There is a fibrous membrane on the inside of the eggshell, which is made up of reticular fibers of protein. Eggshell is a completely highly bound biocalcium composed of a combination of inorganic and organic proteins.

The organic matter in eggshell is thought to affect the nucleation of calcium carbonate crystals, control the formation of crystals, and play an important role in the biological system of eggshell.

Eggshell membrane is a fibrous membrane between egg shell and egg white, close to the papillary layer of eggshell, accounting for about 1.02% of the wet weight of the egg, 0.24% of the dry weight of the egg, thickness of about 70 μm, composed of the outer eggshell membrane, the inner eggshell membrane, and the limiting membrane. As a kind of semi-permeable hydrophilic biological active membrane, eggshell membrane has good air permeability, moisture retention and adsorption properties. The semi-permeable experiment showed that the eggshell membrane could selectively pass through small molecules such as acetic acid and water, but could not pass through large molecules such as glucose. Eggshell membrane is a complex network structure composed of biomolecules and protein fibers. The network structure of eggshell membrane is stable and insoluble in water. The FTR spectra of eggshell membrane showed that the main absorption peaks were located at 3200 ~ 3500 cm, 1651, 1538 and 1384 cm^{-1}, respectively. Thus, the eggshell membrane contains amino ($-NH_2$) and amide groups ($-CO-NH_2$), which make the surface of the eggshell membrane positively or negatively charged at different pH conditions (Xiao, 2009).

2.2 Flash evaporation

Flash evaporation is the phenomenon that the saturated liquid at high pressure enters a relatively low pressure vessel and becomes a part of the saturated vapor and saturated liquid under the pressure of the vessel due to the sudden decrease of the pressure. Miyatake et al. (1972) carried out Flash Evaporation experiments in water with superheat of 3-5 °C, equilibrium temperature (liquid temperature corresponding to equilibrium pressure) of 40 °C, and initial water temperature of 60 °C and 80 °C, respectively. They defined the non-equilibrium fraction NEF and the non-equilibrium temperature difference NETD to better describe the flash process (Miyatake et al., 1975), which is expressed as follows (Equation 1) and (Equation 2):

$$NET(t) = T(t) - T_e / T_0 - T_e \tag{1}$$

$$NETD = T(t^*) - T_e \tag{2}$$

Where,

T(t): Water temperature at time t (°C);

T_e: Temperature at flash equilibrium (°C);

T_0: The initial water temperature (°C).

It is found that the NEF change curve with time can be divided into two processes. The phenomenon in the earlier period of time was intense, and the temperature changed rapidly. The evaporation in the latter period of time still occurred, but the phenomenon and temperature changes were relatively small.

It is found that the liquid level, degree of superheat and initial water temperature have important effects on the flash propagation depth and the non-equilibrium temperature difference NETD, etc. However, at a higher initial temperature, the lower the water level was, the more intense the flash, and the correlation formula between NETD and NEF was given (Miyatake et al., 1972).

Freon-11 was used as the working medium to study the evaporation rate in the process of flash evaporation, and the depressurization rate was controlled between 1.9-6.3Pa/s. It was found that when the superheat of the liquid exceeded 1.5 K, the evaporation rate increased significantly (Clegg & Papadakis, 1986). The propagation mechanism of flash wave was studied more fully with R-12 and R-114 as working medium respectively, and the qualitative characteristics of flash wave were described (Hill & Sturtevant, 1990). For example, the starting position of the flash wave, the delay time of the flash wave, and a self-starting limit pressure exist at the same time. When the pressure is exceeded, the flash wave will not be generated spontaneously. Finally, the wave velocity, pressure and two-phase flow velocity of the flash wave are quantitatively described. When the depressurization rate is fast enough, the vaporized cores present in the liquid are not activated, and the liquid may reach the dynamic limit of superheat before the phase transition occurs, which is close to the maximum limit determined by the random density fluctuations that cause homogeneous boiling (Elias & Chambre', 1993). The spray flash characteristics and heat transfer characteristics of R404A at low saturation temperature were numerically simulated, and the low temperature spray flash mechanism of volatile working substances was revealed (Zhou et al., 2018). The initial flash temperature, ambient pressure and jet velocity are the main factors affecting the flash intensity and jet state (Mutair & Ikegami, 2010). Jet velocity, initial solution temperature and flash pressure are the main factors affecting the state of jet and flash evaporation (Wu et al., 2014).

2.3 Flash separation experiment

Flash separation experiments were performed in a 100 mL stainless steel autoclave batch reactor. As shown in Figure 1, in an experiment run, about 7 g eggshells were added into the reactor. After the reactor was sealed, heated by an electric resistance heater up to the setting experiment pressure. And then open the reactor valve to bring the pressure down to atmospheric pressure instantly. The mixture of eggshells and eggshell membranes remained in the collection vessel were collected, dried, and weighed after each experiment run.

2.4 Analytical methods

Products separation and collection

Eggshells and eggshell membranes were collected separately from mixture by self-made cyclone device (Chi et al., 2019).

In order to determine the ratio of eggshell membranes to eggshells, ten intact eggs were selected and divided into 5 groups with 2 eggs in each group. In order to completely separate the eggshell membranes from the eggshells, the eggshell membranes were peeled artificially. Weighing the eggshell membranes and eggshells after drying. The electronic scale with a precision of 0.01 g was used for weighing (M600TB-B, Shenzhen Meifu

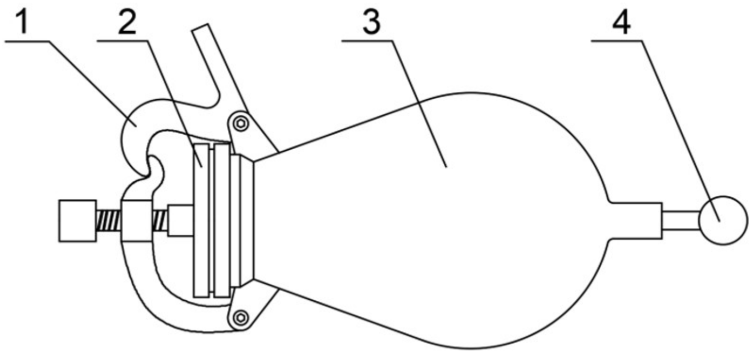

Figure 1. Stainless steel autoclave batch reactor. 1) valve; 2) reactor cap; 3) reactor; 4) pressure gage.

Electronics Co., Ltd., China). The results show that the ratios of eggshell membrane to eggshell in 5 groups were 2.985%, 3.125%, 3.093%, 3.068%, 2.946%, respectively. The mean value was 3.043%. Therefore, the separation rate was expressed as follows (Equation 3):

$$Y = m/0.03043M \times 100\% \quad (3)$$

Where,

Y: The separation rate (%);

M: Eggshell fragment mass (g);

m: Mass of eggshell membrane after experiment (g).

Data treatment and statistical analysis

Data resulting from the factorial designs were evaluated for significance and estimated effects of the main and interaction variables ($p < 0.05$). The differences between the averages of the tests were evaluated by analysis of variance (ANOVA), followed by the Tukey test, with a 95% confidence level.

3 Results and discussion

3.1 The experimental design

The pressure in the container (A, 0.8-1.0 Mpa), moisture content of eggshells (B, 30-50%), eggshells' particle size (C, 5.0-10.0 mm) were selected as test factors. Filtered eggshells with sieves and took the average particle size. The separation rate was taken as the test indicator (Y). Used orthogonal experiment with 3 factors and 3 levels, calculated the separation rate of each group, and analyzed the shadow response of each factor to the test indicator. Table 1 shows the test factor levels.

3.2 Establishment and variance analysis of regression equation

Table 2 shows the results of orthogonal experiment, A, B and C code values for factors.

Used Designer-Expert 8.0.6 quadratic regression analysis of the test results. The regression equation of separation rate was obtained and its significance was tested.

Table 1. Test factor levels.

Level	Factor		
	Pressure (MPa)	Moisture Content (%)	Particle Size (mm)
	A	B	C
1	1.0	50	10
0	0.9	40	7.5
-1	0.8	30	5

Table 3 shows the ANOVA for response surface quadratic model. The order of each factor and its interaction on membrane recovery was A, C, B, A^2, B^2, AB, BC, AC, C^2. The pressure, moisture content, eggshells' particle size, the interaction term of pressure and moisture content, the interaction term of pressure and eggshells' particle size, the interaction term of moisture content and eggshells' particle size, the quadratic term of pressure and moisture content have extremely significant effects on the separation rate ($p < 0.01$). The quadratic term of eggshells' particle size had no significant effect on the separation rate.

The separation rate model was extremely significant ($p < 0.01$), and there was no lack of fit ($p = 0.2292$, $p > 0.05$), which indicated that the model was very significant and the model error was small with practical application significance. The factors affecting the separation rate were pressure > eggshells' particle size > moisture content.

The ternary quadratic regression equation was $Y = 64088 + 2.90A + 2.71B - 2.81C + 0.90AB - 0.75AC + 0.85BC - 2.42A^2 - 2.25B^2$, showing a good fit between the test value and the predicted value, and could be used to predict the separation rate of eggshells and eggshell membranes.

3.3 Interaction analysis

As shown in Figure 2, used Design-Expert software to obtain the response surface of the interaction of various factors.

When the eggshells' particle size was 5 mm, the separation rate had a response peak with a pressure of 1.0 Mpa and with a moisture content of 45%. As the moisture content increased, the separation rate increased to a maximum response value and then dropped. However, the response surface graph of the

Table 2. Results of orthogonal experiment.

Run	Factor			Separation Rate (%)
	A	B	C	
1	1.0	40	10	61.76
2	0.8	50	7.5	59.08
3	0.9	30	5	63.15
4	1.0	50	7.5	66.33
5	0.8	40	10	57.11
6	0.9	40	7.5	64.81
7	0.8	30	7.5	55.89
8	0.8	40	5	61.13
9	0.9	40	7.5	65.22
10	1.0	30	7.5	59.53
11	0.9	50	5	67.29
12	0.9	40	7.5	65.17
13	0.9	50	10	63.28
14	0.9	40	7.5	64.63
15	0.9	40	7.5	64.55
16	0.9	30	10	55.74
17	1.0	40	5	68.78

Table 3. ANOVA for response surface quadratic model.

Source	Sum of Squares	df	Mean Square	F Value	P
Model	247.02	9	27.45	192.22	< 0.0001[a]
A	67.22	1	67.22	470.79	< 0.0001[a]
B	58.70	1	58.70	411.10	< 0.0001[a]
C	63.06	1	63.06	441.62	< 0.0001[a]
AB	3.26	1	3.26	22.82	0.0020[a]
AC	2.25	1	2.25	15.76	0.0054[a]
BC	2.89	1	2.89	20.24	0.0028[a]
A^2	24.64	1	24.64	172.59	< 0.0001[a]
B^2	21.30	1	21.30	149.19	< 0.0001[a]
C^2	0.29	1	0.29	2.02	0.1982
Residual	1.00	7	0.14		
Lack of Fit	0.62	3	0.21	2.21	0.2292
Pure Error	0.38	4	0.094		
Cor Total	248.02	16			

df: degree of freedom. [a]Extremely significant effect ($p < 0.01$).

eggshells' particle size was relatively flat, which was consistent with the results of variation analysis indicating that the effect of pressure was more important than that of the eggshells' particle size.

According to the response surface analysis, the optimized processing parameters of separating eggshell membranes and eggshells with flash evaporation were 1.0 Mpa pressure, 45% moisture content, and 5 mm eggshells' particle size. Based on the practical operability, we carried out three verification experiments. The results showed that the separation rate was 69.16% and showed no significant difference from the predicted value of 69.00%. In summary, the processing parameters for separating eggshell membranes and eggshells with flash evaporation as identified in this work were accurate, reliable, and high practical value.

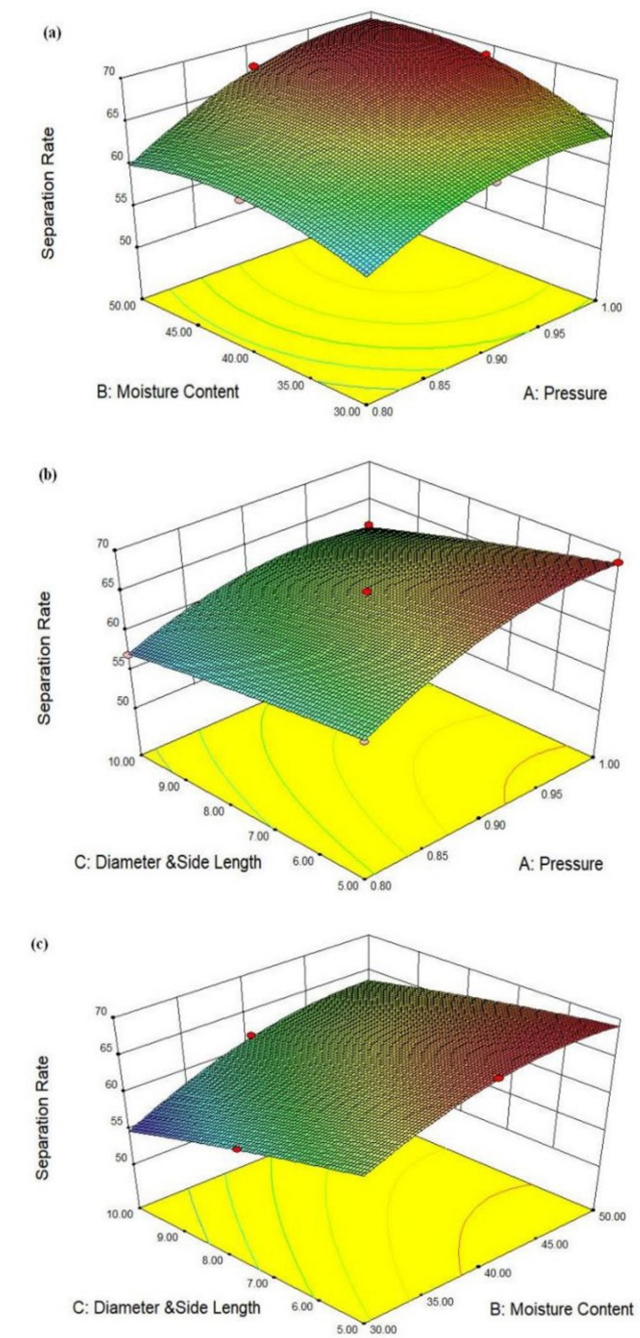

Figure 2. The effect of 3 factors on the separation rate of eggshell membranes and eggshells. (a) The pressure in the container (A) and moisture content of eggshells (B); (b) the pressure in the container (A) and eggshells' particle size (C); (c) moisture content of eggshells (B) and eggshells' particle size (C).

4 Conclusions

Restricted by experimental conditions, the maximum pressure in the reactor could only reach 1 MPa. In the experiments with 1 MPa pressure, the separation rate of eggshell and eggshell membrane by flash evaporation was more than 60%. The experimental analysis shows that if the pressure increased further, the separation rate will increase. The optimized processing parameters of separating eggshell membranes and eggshells with flash evaporation were pressure at 1.0 Mpa, moisture content at 45%, and eggshells' particle size at 5 mm which shows the viability of the flash separation method proposed. Therefore, an efficient and eco-friendly method was developed to separate eggshells and eggshell membranes.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Yuan Chi: Conceptualization (Lead), Investigation (Equal), Data curation (Equal), Writing - review & editing (Lead). Ruihong Liu: Conceptualization (Equal), Data curation (Lead), Investigation (Equal), Writing-original draft (Lead), Writing - review & editing (Equal). Mengmeng Lin: Data curation (Supporting), Investigation (Supporting), Writing - review & editing (Supporting). Yujie Chi: Conceptualization (Supporting), Supervision (Lead), Resources (Lead), Writing - review & editing (Supporting).

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