

Effects of hot-air drying temperature on drying characteristics and color deterioration of rape bee pollen

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ABSTRACT

The effects of different hot-air drying (HAD) temperature (40, 50, 60, and 70 °C) on the drying characteristics, color changes, the contents of α -dicarbonyl compounds (α -DCs), 5-hydroxymethyl furfural (5-HMF) and carotenoids of rape bee pollen were investigated in the study. The results showed that increasing the drying temperature from 40 to 70 °C shortened the drying time by 65 %. HAD caused lower L^* and b^* values, as well as higher a^* values. Browning index and 5-HMF content increased with increasing drying temperature. The relative content of antheraxanthin increased 230 % at 70 °C while lutein and zeaxanthin decreased by 74 and 81 % than that of fresh (non-heated) pollen. The contents of 3-deoxyglucosone, 1-deoxy-2,3-pentosulose, antheraxanthin, and lutein were related to the color deterioration in HAD process in rape bee pollen. This work is of great practical significance to provide scientific basis for quality optimization of bee pollen in the drying process.

1. Introduction

Today, the high demand of consumers for naturally plant-based food is driving the development of food industry to provide foods with rich nutritious and functional properties. Bee pollen has attracted great attention in food industry since it is a valuable source of nutrients (Borel, Marques, & Prado, 2020). It contains a large number of bioactive compounds, including proteins (10–40 %), carbohydrates (13–55 %), lipids (1–13 %), fibers (0.3–20 %) and various microelements like minerals, polyphenols, carotenoids and vitamins, thus preventing a variety of diseases, such as reduce the risk of cancer, as well as cardiovascular and neurodegenerative diseases (Lu et al., 2022; Végh et al., 2021; Wang et al., 2022). However, the fresh bee pollen contains 21 to 30 % of the water and its properties initiate to degrade just after the collection (Wang et al., 2022; Kieliszek et al., 2018; Thakur & Nanda, 2020). In addition, the high level of moisture content is a good media for the growth of microorganisms (yeast, mold and spoilage bacteria) and makes bee pollen susceptible to mildew and other degradation reactions that lead to the reduction of active substances and loss of nutrients (Kanar & Mazi, 2019). Therefore, it should be dehydrated until 5–8 % (wet basis) for commercial purposes (Zuluaga-Domínguez, Serrato-

Bermudez, & Quicazán, 2018). Water activity of bee pollen that is ready for consumption is about 0.261–0.28 after dehydration (Kieliszek et al., 2018). Drying the pollen prevents spoilage of the product by reducing the content of free water responsible for spoilage reactions. It may also extend its shelf life by inhibiting the growth of microorganisms and biochemical reactions (Ni et al., 2022). Undoubtedly, drying is a commonly used technology for enhancing the stability of bee pollen products.

Hot-air drying (HAD) is widely used in industrial drying fields for the dehydration of food and agricultural products due to its lower risk of microbial contamination, low investment cost and better control of drying conditions (Isik, Ozdemir, & Doymaz, 2019). It is also one of the most common methods used for the preservation of bee pollen (Kanar & Mazi, 2019). HAD can be beneficial for bee pollen as it carries water away between pollen grains and reduces pollen grain adhesion (Lewicki, 2006). However, inadequate drying conditions could seriously degrade the color of rape bee pollen during the drying process (De-Melo et al., 2016). A previous study reported that the bee pollen dried at 40, 45, 50, 55 and 60 °C were characterized by the lower L^* and b^* values compared to that of the fresh ones. Additionally, the bee pollen samples dried at 40 °C were characterized by the lowest values of total color difference

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(ΔE) (Isik et al., 2019).

Color is one of the most important features of the appearance of bee pollen and affects consumer acceptance (De Jesus Inacio et al., 2020). Pollen grains can have different colors, including yellow, grey-white, orange, reddish, greenish or blue, that indicate the variety of plant species from which the pollen was collected. As reported above, the color of bee pollen is also influenced by the processing conditions and the presence of pigments (De Jesus Inacio et al., 2020). It is well known that the Maillard reaction has been identified as the main non-enzymatic browning reaction that often occurs during the food drying process (Aktağ et al., 2021). It is significantly involved in the formation of food pigments (melanoidins) and can also produce numerous intermediates, including some potentially toxic compounds, such as α -DCs and 5-HMF (Kwon, Ahn, & Lee, 2021). The α -DCs are generated by the decomposition of sugar or other Maillard reaction products, which are identified as highly reactive products in the Maillard reaction (Kwon, Ahn, & Lee, 2021). In the Maillard reaction, Schiff bases are initially formed through the condensation process of primary amines and active carbonyl groups, and then, Amadori/Heyns products generate the DCs by removal of the amine. Following the formation of intact DCs, *retro*-aldol reactions, fragmentation, and water elimination reactions lead to the generation of shorter chain DCs such as methylglyoxal (MGO) (Aktağ & Gökmen, 2021). The α -DCs play an important role in the formation of toxicogenic compounds such as furan derivatives and advanced glycation end-products (AGEs), which may cause health risks such as diabetes, cataract, and Alzheimer disease (Gürsul Aktağ & Gökmen, 2020). Importantly, the α -DCs are essential precursors of coloring compounds (Chen & Kitts, 2011). It has been reported that the α -DCs are the direct precursors for carbohydrate-based melanoidins (Feng et al., 2021). Recently, α -DCs have been shown to be key precursors to the color formation during the storage of apple juice (Paravisini & Peterson, 2018). As a typical Maillard reaction product, 5-HMF formed during thermal processing of foods in the initial stages of the Maillard reaction may serve as an indicator of pollen deterioration. It is reported that high concentrations of 5-HMF from food have an adverse effect on human health, such as irritation of mucous membranes, skin, upper respiratory system, and eyes, as well as neurodegenerative, diabetes, and cardiovascular diseases (Martins et al., 2022). The formation of 5-HMF is associated with the browning of persimmon samples exposed to heat applications and HAD samples are characterized by the lowest lightness index and the highest amount of 5-HMF (Kayacan et al., 2020). To the best of our knowledge, only few studies in bee products have compared the differences of α -DCs between naturally matured and artificially heated acacia honey, and composition of α -DCs in propolis from different plant origins (Yan et al., 2019; Song et al., 2021). Furthermore, no information is currently available on the effects of different drying temperatures on the content of α -DCs and 5-HMF of bee pollen.

In addition to the effect of browning on the color of bee pollen during drying process, the degradation of pigments like carotenoids is another key factor affecting the color deterioration. The diverse color of pollen is mainly determined by the composition and content of natural plant pigments (carotenoids and anthocyanins), which are varied by factors such as the botanical sources, geographic origin and harvest season (Gardana et al., 2018; Sattler et al., 2015; Thakur & Nanda, 2020; De Jesus Inacio et al., 2020). Genetic and biochemical composition analyses showed no significant difference in total phenol content between white and orange pollen, while carotenoid content in orange pollen was significantly higher than that in white pollen (Wakelin, Lister, and Conner, 2003). It has also reported that the color of bee pollen is also influenced by the presence of carotenoids like lutein and β -carotene (De Jesus Inacio et al., 2020). The main carotenoids identified in bee pollen have been reported to be α - and β -carotene, cryptoxanthin, zeaxanthin, neoxanthin, and lutein (Salazar-González et al., 2020). According to Margaoan et al. (2014), lutein was the major carotenoid found in Romania pollen samples, with concentrations ranging from 57 to 476 $\mu\text{g/g}$ (dry basis). These carotenoids have a wide range of physiological

functions like lower the risk of different types of cancer or cardiovascular disease and age-related diseases of the eye (Meléndez-Martínez & Mapelli-Brahm, 2021). However, they can easily degrade in the presence of high temperature and light (Wani et al., 2020). These factors promote color changes due to the rearrangement or formation of degrading compounds such as *cis*-isomers (like 5,8-epoxyderivatives), epoxides (like apocarotenones and apocarotenals) or short-chain products (like 5,6-epoxy- β -ionone, ionene, β -cyclocitral, β -ionone) (Pénicaud et al., 2011). Previous study reported that carotenoids can be more or less affected depending on the heat treatment time and temperature, leading to an increase or decrease of their amounts (Murador et al., 2014). This may be on account of the difference in processing method, the type of food matrix and carotenoids structure that affected the content changes of carotenoids. Hence, the changes in the content of carotenoids are necessary for the assessment of the color deterioration of bee pollen.

Therefore, the aim of this study was to evaluate the drying characteristics and changes of color (the formation of Maillard reaction products and the degradation of pigments) of rape bee pollen during hot-air drying process. The color transformation mechanisms in drying process were carried out via quantitative analysis of α -DCs, 5-HMF, carotenoids. Additionally, the correlations between chemical compounds and browning index formed in the rape bee pollen samples were established. The results of this study provide an insight into the drying characteristics and color transformation mechanism of rape bee pollen, and provide scientific basis and guidance to improve the quality of dried bee pollen.

2. Materials and methods

2.1. Materials

Fresh rape bee pollen, characterized by its intense yellow oblate granule, were obtained from Gangzhou Village, Xiaosi Township, Caidian District, Wuhan City, Hubei Province, China (30°31'N, 113°90'E). All the samples were sealed and stored in a refrigerator at -18 °C before drying. The initial moisture content of the samples was $25.24 \pm 0.68\%$ (wet basis). The moisture content was determined according to the method described in the literature (Song et al., 2020).

2.2. Drying procedure

The single layer of fresh rape bee pollen samples was spread evenly on the feeder tray (200 × 150 × 10 mm). The thickness of the layer was 8 mm with an average weight of 200 ± 5 g. HAD was carried out at 40, 50, 60, and 70 °C in the oven (DHG-9123A, Shanghai Jinghong Experiment Facility Co., Ltd., Shanghai, China) and air velocity of 1 m/s at the bee pollen surface. Samples were taken at 20 min intervals until the samples reached a final moisture content of approximately 6 % (wet basis). After drying, the samples were taken out and cooled to room temperature. Then, they were put in a sealed polythene bag and stored in a desiccator at room temperature.

The moisture ratio (MR) was calculated using the following formula (Song et al., 2020):

$$MR = M_t/M_0 \quad (1)$$

where, M_0 indicates the initial moisture content of the bee pollen (dry basis), and M_t indicates moisture content (dry basis) at a specific drying time t .

The drying rate (DR) was calculated using the following formula (Song et al., 2020):

$$DR = (M_{t1} - M_{t2})/(t_2 - t_1) \quad (2)$$

where, M_{t1} and M_{t2} indicate the moisture content of the material (dry basis) at the drying time t_1 and t_2 , respectively.

2.3. Measurement of color parameters and indices

The color measurements of the dried rape bee pollen were carried out using a CM-5 spectrophotometer (Konica Minolta, Japan) according to the CIE $L^*a^*b^*$ scale. Based on the three color parameters, the total color difference (ΔE) between the fresh and dried samples was calculated according to the formula (Wang et al., 2017):

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (3)$$

where, L_0^* , a_0^* , and b_0^* indicate the lightness, redness, and yellowness of fresh rape bee pollen; while L^* , a^* , and b^* indicate the lightness, redness, and yellowness of dried rape bee pollen.

The browning index (BI) was calculated using the following formula (Verma & Yadav, 2022):

$$BI = 100 \times (X - 0.31)/0.17 \quad (4)$$

$$X = [(a^* + 1.75L^*)a^*]/(5.645L^* + a^* - 3.012b^*) \quad (5)$$

where, L^* , a^* , and b^* indicate the lightness, redness, and yellowness respectively of dried rape bee pollen.

Each sample was tested six times and the average value was taken.

2.4. Identification and quantification of α -DCs in rape bee pollen

The extraction and derivatization of α -DCs in rape bee pollen was based on a published method with slight modifications (Yan et al., 2019). Fresh bee pollen samples were finely ground and then stored at 4 °C until analysis. The mass of 1.0 g of ground bee pollen was added into a 15 mL centrifuge tube and extracted with 5 mL of 10 % methanol. Centrifugation was performed at 10000 \times g at 30 °C for 5 min. The volume of 2 mL of supernatant was mixed with 1 mL of 0.8 % o-phenylenediamine (OPD). Then, the tube was shaken without heating until complete dissolution, and derivatized for 12 h in an orbital shaker at the temperature of 30 °C. The reaction solution was purified using a C18 solid-phase extraction column (6 cc, 500 mg, Waters, Milford, Massachusetts USA). The eluent was dried with nitrogen and redissolved with methanol. The solutions were filtered using 0.2 μ m nylon membrane. The filtrate was collected in a 1 mL autosampler vial for further analysis.

Quantitative analysis of α -DCs were carried out according to a published procedure (Song et al., 2021) with minor modifications. The α -DCs were determined by using an Agilent 1290 UHPLC system coupled via electrospray ionization to an Agilent 6470A QQQ MS (Santa Clara, CA). Agilent ZORBAX Eclipse Plus C18 (2.1 \times 100 mm, 1.8 μ m) column was selected and the column temperature was set at 45 °C. The detection method employing ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometry in MRM mode was then used to quantify α -DCs in rape bee pollen samples. Samples were eluted using a step-wise gradient of 0.1 % formic acid aqueous solution (A) and methanol (B) as follows: 10–20 % B (0–1 min), 20–45 % B (1–10 min), 45–95 % B (10–12 min), 95 % B (12–15 min), 95–10 % B (15–15.1 min), followed by equilibration at 10 % B for 4 min. The flow rate was 0.3 mL/min. The MS was operated in electron spray ionization (ESI)-positive ion mode with a capillary voltage of 3500 V and nozzle voltage of 500 V, nebulizer pressure was 45 psi, drying gas temperature was 250 °C and drying gas (N_2) flow rate of 7 L/min, sheath gas temperature was 350 °C and its flow rate was 11 L/min. A volume of 2 μ L sample filtrate was injected for each run.

2.5. Extraction and quantification of 5-HMF in rape bee pollen

5-HMF was extracted from rape bee pollen samples using the method described by Kanar & Mazı (2019). The mass of 10.0 g of ground rape bee pollen was added into 50 mL centrifuge tube, 15 mL of methanol was added and vortex to mixed well. The mixture was then sonicated for 5

min and then centrifuged at 10000 \times g for 30 min. The collected supernatant was used for HPLC analysis.

The 5-HMF content of samples were carried out according to Yan et al. (2019). The HPLC 1260 system (Agilent Technologies, U.S.A.) was equipped with Agilent SB-C18 analytical column (50 \times 4.6 mm, 5 μ m) and an ultraviolet detector (285 nm). Isocratic elution was performed (water: methanol, 90:10) at a flow rate of 0.3 mL/min for 13 min at 50 °C. A volume 5 μ L filtrate was injected for each run.

2.6. Extraction and quantification of carotenoids

The extraction of carotenoids in rape bee pollen was based on the method of a previous study with slight modifications (Lux et al., 2019). The mass of 2.00 \pm 0.05 g of rape bee pollen samples were ground with 1 mL of water and then homogenized. The procedure was performed under dim light. The samples were extracted with 10 mL of methanol/ethyl acetate/petroleum ether (1:1:1, v/v/v) containing butylated hydroxytoluene, 0.1 % (w/v) to protect the carotenoids from degradation. Each extraction process was repeated twice with 10 mL of the aforementioned solvent. After adding 1 mL of methanol, the combined organic layer was washed twice with 3 mL of water. After evaporation of the solvent with a gentle stream of nitrogen, the carotenoids were dissolved in 1 mL of isopropanol/trichloromethane (70:30, v/v) and filtered using 0.2 μ m nylon membrane filter for further analysis.

All measurement were performed by using an Agilent 1260 Infinity LC System (Agilent Technologies, U.S.A.) including a vacuum degasser (Serial No. JP82009779), a binary pump (Serial No. DE63056273), an auto-sampler (Serial No. DE82555638), a thermostated column compartment (Serial No. DE90359987) and a diode-array detector (Serial No. DEAA301873). YMC Carotenoid (250 \times 4.6 mm I.D.S-5 μ m) column was selected for better separation of the samples with sharp, symmetrical peaks and the column temperature was set at 60 °C. Samples were eluted using a step-wise gradient of 75 % methanol aqueous solution (A) and isopropanol/methanol (50:50, v/v) (B) as follows: 0–12 min, 45 % A; 12–5 min, 0 % A; 15–40 min, 0 % A and 40–41 min, 45 % A at a flow rate of 1.2 mL/min. Carotenoids were monitored at 428 nm, 444 nm, 446 nm, 450 nm, 451 nm, 472 nm and 501 nm, respectively, recording additional UV/Vis spectra in the range of 230–630 nm. A volume of 40 μ L filtrate was injected for each run. The carotenoid content was calculated as a percentage value of fresh samples. The carotenoid content was expressed by mean values \pm standard deviations of three replicates.

2.7. Statistical analysis

One-way analysis of variance (ANOVA) and Duncan's multiple range test at 5 % probability level was conducted to determine the differences between samples. Statistical analysis was run using SPSS 26.0 (SPSS Inc., Chicago, IL, USA) and Origin Pro 2021 (OriginLab Inc., Northampton, MA, USA) softwares. Two-tailed Pearson correlation test was conducted to determine the correlations between color parameters, α -DCs and carotenoids. All analyses were performed in triplicate, and the results were expressed as the mean \pm standard deviation.

3. Results and discussion

3.1. Analysis of drying characteristics

Drying temperature and time both play a significant role in the moisture content of food. The HAD drying kinetics of rape bee pollen samples dried under different drying temperature (40, 50, 60, and 70 °C) are illustrated in Fig. 1a. The total drying time of rape bee pollen significantly decreased with the increase in drying temperatures and it was 47, 52 and 65 % shorter at 50, 60 and 70 °C than that at 40 °C, respectively (Fig. 1b). Meanwhile, HAD time decreased by 43, 33 and 16 % for each 10 °C when the drying temperature increased from 40 to 50,

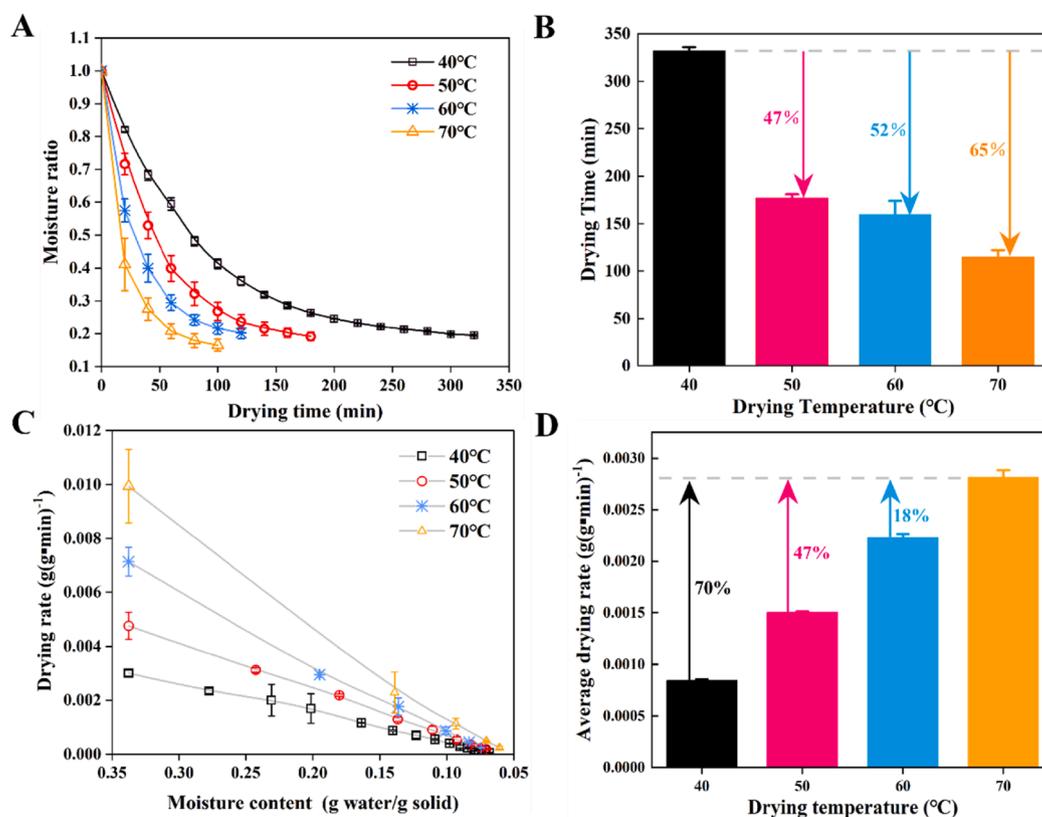


Fig. 1. HAD characteristics of rape bee pollen. (A) drying kinetic curves of rape bee pollen samples under different drying temperature; (B) reduction in drying time under different drying temperature of rape bee pollen until reaching a moisture content of 6% in wet basis; (C) drying rate curves of rape bee pollen samples under different drying temperature; (D) reduction in the average drying rate under different drying temperature of rape bee pollen.

60 and 70 °C. This phenomenon appeared to occur because the higher drying temperature accelerated the mass transfer rate between the drying air and the samples, as well as the diffusion of water at an early stage of drying (Wang et al., 2017). It has also been reported that the lower HAD temperature requires longer drying time for lotus bee pollen (Song et al., 2020).

The relationship between the moisture content (dry basis) and drying rate of rape bee pollen under different drying temperatures is shown in Fig. 1c. The difference in the falling drying period in rape bee pollen samples was huge at different drying temperatures. The drying rate decreased significantly as the moisture content decreased. The average drying rates were reduced by 70, 47 and 18 % under the temperature of 40, 50 and 60 °C compared to that at 70 °C, respectively (Fig. 1d). Such a big difference was related to the moisture gradient driven by the drying temperature. For the samples under the same drying temperature, the drying rate was faster in the early stage while slower in the later stage. It was probably due to the migration and evaporation of a large amount of free water in the early stage of HAD, and then the loss of weakly bound water in cytoplasm at the later stage of HAD. It is reported that free water can be vaporized directly first whereas the bound water had very restricted mobility due to hydrogen bonding with the cytoplasm (Gezici-Koç et al., 2017; Wang et al., 2018). At the later stage of drying, along with the decrease in moisture inside the material and hardening of the rape bee pollen surface, the diffusion of moisture was influenced by external and internal resistances, therefore the drying rate decreased slowly until equilibrium was achieved and drying was stopped. When the moisture transfer rate inside the samples was lower than the surface evaporation rate, the samples were found to be at a falling drying stage, indicating that the drying process was guided by moisture diffusion (Mirzaei-Baktash et al., 2022).

3.2. Color measurement

Color is among the most significant sensory evaluation indicators for most foods. Undesired color changes have an adverse effect on their quality. The effect of different HAD temperature on the surface color parameters L^* , a^* , b^* , ΔE and BI indices of rape bee pollen are shown in Fig. 2. Compared to fresh rape bee pollen, the HAD samples significantly decreased the L^* and b^* values, and increased the a^* values ($P < 0.05$). The L^* values changed from 64.51 to 57.11 with the drying temperature increasing from 40 to 70 °C (Fig. 2a). The results show that the lightness of rape bee pollen significantly decreased during HAD, and the dried rape bee pollen were characterized by the significantly darker color than the fresh ones. It can therefore be concluded that the higher drying temperature caused a significant deterioration in the color of rape bee pollen, which can be attributed to the degradation and isomerization of carotenoid pigment associated with the Maillard reaction (Montoya-Ballesteros et al., 2014). Most probably, the alteration or loss of carotenoids pigments during drying caused the surface color fading through geometric isomerization and enzymatic or non-enzymatic oxidation. The results are in line with the published data (Multari et al., 2018). In addition, the decrease of L^* value with increasing HAD temperature was also influenced by the formation of brown products that turned rape bee pollen dark brown. The water in the bee pollen diffused outwards as the drying process progressed, the apertures of the bee pollen wall fold inwards due to the shrinkage of the pollen surface, resulting in the reduction of light reflection (Katifori et al., 2010).

The a^* values of the samples increased from 2.53 to 5.60 at the drying temperature from 40 to 70 °C, while the b^* values showed the opposite tendency, and decreased from 52.75 to 49.08 (Fig. 2a). The values of ΔE index of rape bee pollen increased from 1.58 to 8.89 with increasing the drying temperature from 40 to 70 °C. The results show that the lower temperature favored the color of rape bee pollen, and the

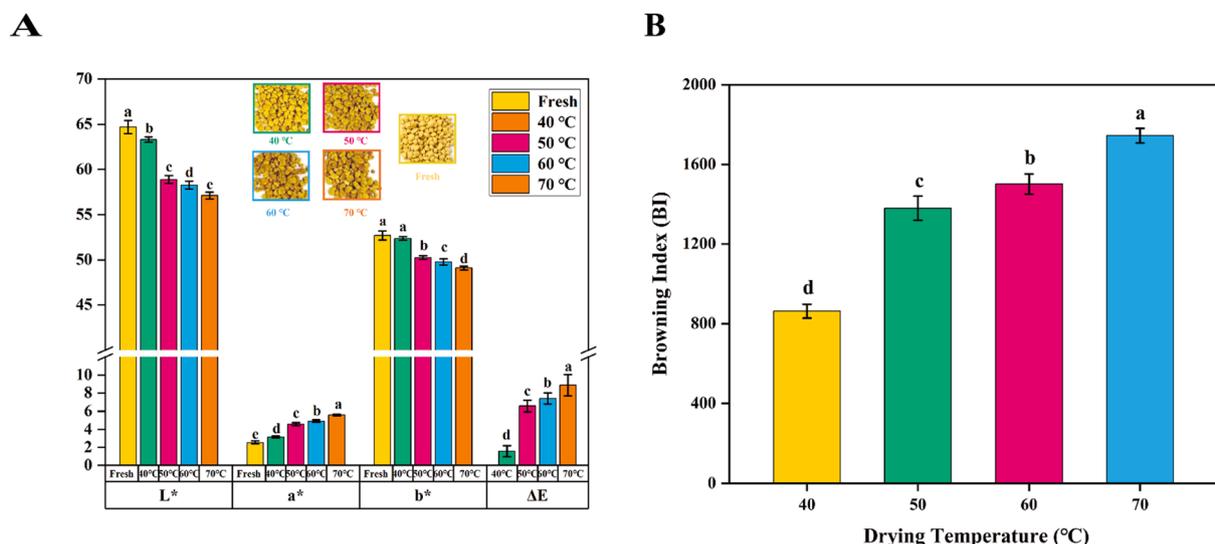


Fig. 2. The color parameters and indices of rape bee pollen subjected to HAD at different drying temperatures: (A) the color parameters L^* , a^* , b^* , and ΔE index of rape bee pollen at different drying temperature.; (B) BI of rape bee pollen at different drying temperatures.

higher drying temperature had an undesirable effect on the sample color and caused a noticeable change in dried bee pollen color. BI value is often used as a necessary parameter to measure the purity of the brown color in the process of non-enzymatic browning take place (Zambrano-Zaragoza et al., 2014). In this study, the values of the BI increased from 861.96 to 1744.29 at the drying temperature from 40 to 70 °C (Fig. 2b). The results indicated that the rape bee pollen samples turned redder during HAD and drying temperature had a significant influence on the BI values. The main reason of the changes in the surface color parameters of rape bee pollen was the fact that bee pollen is characterized by the high content of protein and reducing sugar, and the long drying time and high temperature could favor the Maillard reaction and generate α -DCs and HMF. It can lead to the formation of a brown nitrogen-containing polymer, turning the samples into a deeper red color (Song et al., 2020). On the other hand, the decrease in b^* values was mainly due to the fact that the high temperature induced cell wall disruption and caused the flow of plastids to the intercellular space, increasing the oxidation and degradation of own pigments (Liu et al., 2015). Therefore, in order to further understand the browning mechanism of rape bee pollen during HAD process, it is necessary to study the formation of brown substances and the degradation of pigment in rape bee pollen.

3.3. Analysis of α -DCs

The samples were directly infused into the Q-TOF using iterative MS/MS acquisition mode. The α -DCs were unambiguously identified according to their mass and production ions in agreement with published structural data (Song et al., 2021). Nine different α -DCs including glucosone (GS), methylglyoxal (MGO), 2,3-pentosone (2,3-PS), 3-deoxyglucosone (3-DG), 1-deoxy-2,3-pentosulose (1-DP), 1,4-dideoxyglucosone (1,4-DDG), 1,4-dideoxy-2,3-diketopentose (1,4-DDP), 2,3-butanedione (2,3-BD) and 3,4-dideoxyglucosone-3-ene- (3,4-DGE) have been identified in the rape bee pollen samples. The MRM information on the 9 α -DCs was presented in Table S1. The standard curve for quantitative analysis had a linear relationship in the range of 5–1000 ng/mL, with all of the correlation coefficients (R^2) greater than 0.998. LOD was 0.002–0.075 mg/kg, and LOQ was 0.005–0.230 mg/kg. The average recovery test results ranged from 80 to 103 %.

As shown in Fig. 3, GS was the dominant α -dicarbonyl compound in the rape bee pollen, followed by 3-DG and MGO, while the others were found only in trace amounts. Previous research has shown that GS is formed from the oxidation of sugars catalyzed by transition metal ions

and/or oxidation of Amadori product by hydrolysis and undergo C1-C2 bond cleavage to yield D-ribulose in 37 °C (Zhang & Serrianni, 2012). It is a degradation product and it facilitates the formation of advanced glycation end-products. The highest content of GS was noted for HAD at the temperature of 40 °C. Then, the content of GS decreased gradually with the increase in HAD temperature from 40 to 70 °C. This change may be due to the high volatility in the early Maillard reaction. Additionally, when the temperature exceeds a certain turning point, GS begins to degrade and promotes the formation of advanced glycation end-products (Yan et al., 2019). The content of 3-DG showed a clear increasing trend along with an increase in the initial HAD temperature. Compared with fresh samples, the content of 3-DG increased more than two times at the HAD temperature of 70 °C. This may be due to the acidic condition favored 1,2-enolization to produce 3-DG and the higher temperature accelerated the decomposition of 1,2-enediol (Chen & Kitts, 2011). Meanwhile, due to its relatively stable characteristics, the amount of 3-DG was greater than the degradation in the heating process, so the content increased continuously (Hong & Betti, 2016).

MGO is a decomposition product formed by the antihydroxialdehyde of the intermediate product 3-DG during Maillard reaction and lipid oxidation. The changes in MGO content with HAD temperature increased was comparable to the change in 3-DG. The results were in line with a previous study in which the 3-DG was accumulated faster above the temperature of 50 °C and the effect of 3-DG on color was greatly enhanced in the presence of MGO (Zhang et al., 2019).

A threefold increase in the content of 2,3-BD in rape bee pollen was observed during HAD at the temperature of 70 °C compared to fresh sample, which may be produced by a C2/C4 cleavage of the D-glucose moiety, and it has been detected in beer and honey (da Silva et al., 2015; Marceau & Yaylayan, 2009). 3,4-DGE was gradually degraded at the HAD temperature below 60 °C, which was attributable to the high dicarbonyl reactivity of the α , β -unsaturated carbonyl (Yan et al., 2019). 2,3-PS content dropped rapidly from 1.2 μ g/g in fresh samples to 0.6 μ g/g at the HAD temperature of 40 °C. It remained relatively stable as the temperature increased. A similar trend was also observed in 1,4-DDP. The content of 1-DP significantly increased with increase in the HAD temperature from 40 to 70 °C. There are only few reports on 2,3-PS, as well as 1-DP and 1,4-DDP. Then, the factors affecting their formation require further research.

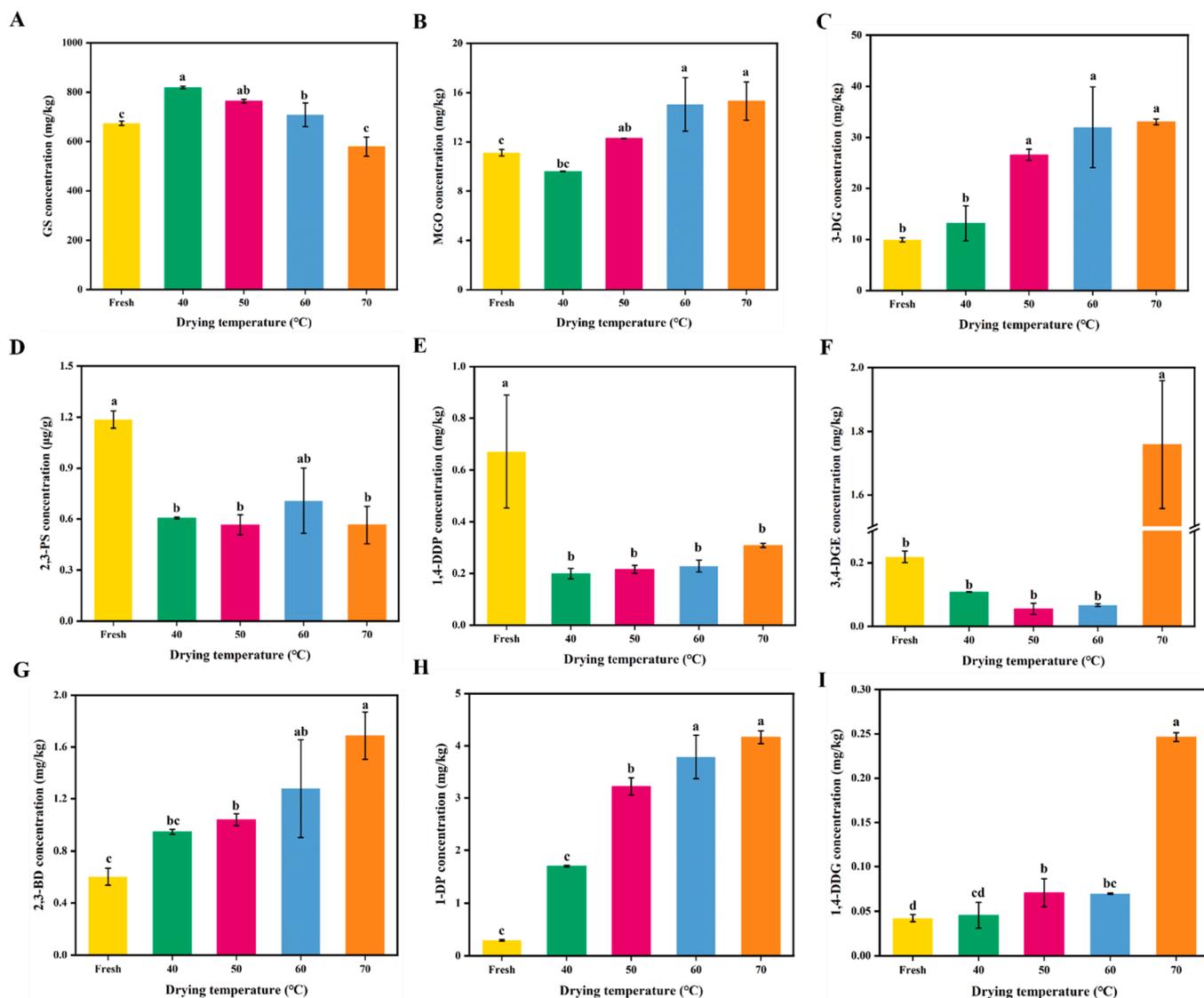


Fig. 3. The influence of HAD temperature on the content of α -DCs of rape bee pollen. (A):GS; (B):MGO; (C):3-DG; (D):2,3-PS; (E):1,4-DDP; (F):3,4-DGE; (G):2,3-BD; (H):1-DP; (I):1,4-DDG.

3.4. Analysis of 5-hydroxymethylfurfural

5-HMF is one of the most known by-products after heating and it is currently recognized as an important quality parameter for some foods that contributes to desired color of caramel after further undergo a series of polymerization reactions (Basaran et al., 2022; Hong & Betti, 2016). HAD temperature and time had a significant influence on the formation of 5-HMF. As is shown in Fig. 4., the content of 5-HMF in fresh rape bee pollen was 0.06 $\mu\text{g/g}$, while it increased to 0.98 $\mu\text{g/g}$ at the HAD temperature of 70 °C. During dehydration of agri-food products, 5-HMF was proposed to be formed mainly during Maillard reactions from 3-DG or 3-DGal via the formation of (Z)-3,4-dideoxyglucosone ((Z)-3,4-DGE), which after 1,2 enolization, dehydration and cyclization reactions from sugar caramelization and Amadori product degradation under acidic conditions (Navarro & Morales, 2017). It has been reported that the 3-DG was formed more rapidly at lower temperatures, while 5-HMF was formed faster at higher temperatures (Arena et al., 2011), which results are consistent with our study. Importantly, the results of α -DCs and 5-HMF confirmed that the occurrence of Maillard reaction may result in the formation of dark pigments and the increase of a^* values and the decrease of L^* values.

3.5. Analysis of carotenoids

As precursors of vitamin A, carotenoids in food not only play an essential role in promotion of health and reduction in risk of eye disease and some forms of cancer, but also are responsible for the color (Meléndez-Martínez & Mapelli-Brahm, 2021). These natural colorants can provide yellow, red and orange color for a variety of foods, including fruit, vegetable and other agricultural commodities. However, the diversity of carotenoids is an essential factor leading to differences in the composition and concentration of each carotenoid, which caused by multiple factors such as the area of collection as well as diversity of floral species (Salazar-González et al., 2020). The three carotenoids, i.e. lutein, zeaxanthin and antheraxanthin have been tentatively identified in the rape bee pollen samples by analyzing the peak order and spectral characteristics (Table S2) (Lux et al., 2019). The carotenoids in the samples were mainly composed of yellow carotenoids. It has been reported that xanthophylls, such as antheraxanthin, lutein and zeaxanthin are the dominant pigments identified in bee pollen, which have an important effect on the color of rape bee pollen (Salazar-González et al., 2020).

It is well known that carotenoids are susceptible to deterioration in the yellow color and the nutritional quality during thermal processing.

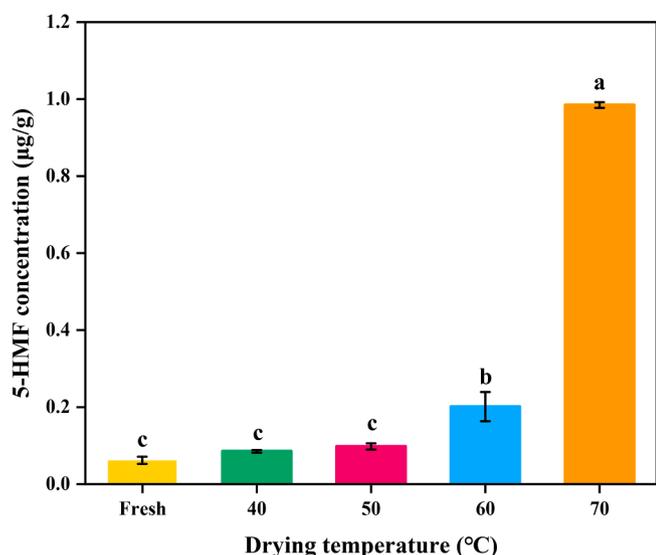


Fig. 4. The influence of HAD temperature on the content of 5-HMF of rape bee pollen.

As shown in Fig. 5, HAD had very similar effects on lutein and zeaxanthin levels. Compared to the fresh sample, about 74 and 81 % of lutein and zeaxanthin in rape bee pollen were lost during HAD at 70 °C, respectively. The levels variation of lutein and zeaxanthin further demonstrated the above color measurement results that the L^* and b^* decreased with increasing the drying temperature. The high drying temperature led to the instability and loss of carotenoids of rape bee pollen in the process of HAD. It can be attributed to the oxidation reactions due to a large number of double bonds in lutein and zeaxanthin that are prone to oxidize (Shen et al., 2015). Furthermore, cell walls constitute an important physical structural barrier controlling the release of carotenoids. The high drying temperatures resulted in the disruption of the integrity of the matrix and modification of the structure of cell membranes and cell walls, facilitating the release of pigments from plant tissues, but the decrease of moisture content had a serious impact on the stability of carotenoids, causing the degradation of carotenoids (Murador, da Cunha, & de Rosso, 2014). In addition, the longer exposure to oxygen and thermal treatment may result in high isomerization of carotenoids (Multari et al., 2018). There was a noticeable increase in antheraxanthin content as a result of HAD. In particular, the

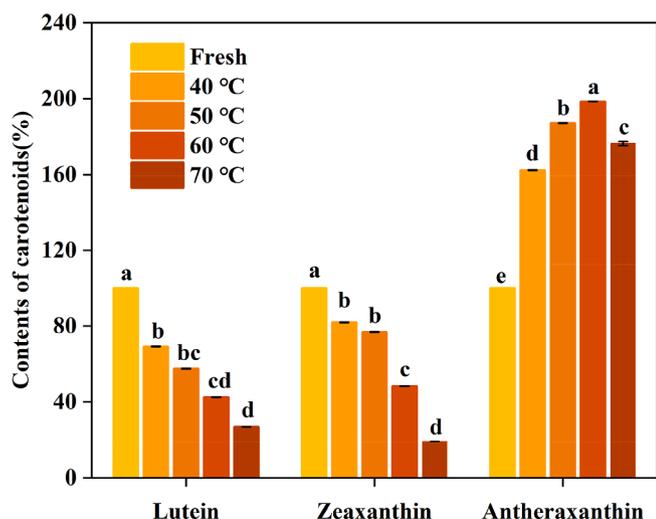


Fig. 5. The effects of HAD conditions on the relative contents of carotenoids of rape bee pollen.

antheraxanthin content of the rape bee pollen dried at 60 °C was almost doubled compared to the fresh samples. This was probably due to the transformation of zeaxanthin as the epoxidation of antheraxanthin was terminated under the catalysis of zeaxanthin epoxidase. However, the identification of pairs of enantiomers and isomers should be subject to a further study by chiral chromatography.

To sum up, the levels variation of carotenoids further confirmed the changes of b^* value with drying temperature increased. The loss of major pigments of rape bee pollen were lutein and zeaxanthin (74 and 81 %), which were responsible for yellow color reflection.

3.6. Correlation analysis

The correlations between color, α -DCs, 5-HMF and carotenoids of rape bee pollen dried under different HAD conditions was shown in Fig. 6. This analysis was performed to understand the effects of different components on pollen color during HAD. Compared to other α -DCs, 3-DG, 1-DP showed positive correlations with a^* values, ΔE and BI indices, and negative correlations with the L^* and b^* values ($P < 0.05$). This agrees with previous studies that the degradation in carotenoids and non-enzymatic browning correlated with lower L^* values significantly (Kayacan et al., 2020). Significant correlation between 3-DG and color formation have been previously reported suggesting their key role in orange juice browning (Paravisini & Peterson, 2019). Furthermore, the α -DCs produced by the Maillard reaction during rape bee pollen drying process is known to be mainly involved in the formation of brown pigments, causing elevated the a^* values, ΔE and BI indices. Among the carotenoids, antheraxanthin and lutein have a significant effect on color of the rape bee pollen samples. Antheraxanthin showed significant positive correlations with parameter a^* , ΔE and BI indices and negative correlations with parameters L^* and b^* ($P < 0.05$). Negative correlations between the content of lutein and parameter a^* have been found in the present study ($P < 0.05$). From our results, the formation of antheraxanthin and some major α -DCs like 3-DG, and the degradation of lutein are mainly responsible for the decrease of L^* and b^* parameters and the increase of a^* parameter. Therefore, the differences in the a^* and b^* values mainly influenced the color variability of the rape bee pollen surface.

4. Conclusion

HAD temperature had a significant effect on drying characteristics and color deterioration of rape bee pollen. The drying time was reduced by 65 % when the drying temperature increased from 40 to 70 °C. Compared to fresh rape bee pollen, increasing the temperature of HAD resulted in a significant decrease in the values of L^* and b^* parameters, as well as a significant increase in the values of a^* parameter. The values of BI index increased more than two times when HAD temperature increased from 40 to 70 °C. The content of GS decreased while the content of 3-DG, MGO and 1-DP increased with the HAD temperature increased. The relative content of antheraxanthin increased 230 % at 70 °C while lutein and zeaxanthin decreased by 74 and 81 %, respectively. The content of antheraxanthin, 3-DG and 1-DP showed positive correlations with parameter a^* , ΔE and BI indices, as well as negative correlations with the values of parameter L^* and b^* ($P < 0.05$). The results demonstrated that the formation of antheraxanthin and 3-DG and 1-DP produced by Maillard reaction as well as the lutein degradation were the main reason that influenced the color deterioration of rape bee pollen in the process of HAD. This study provides a scientific basis for optimizing and regulating the quality of bee pollen during drying process.

CRediT authorship contribution statement

Yan-Xiang Bi: Conceptualization, Methodology, Software, Writing – review & editing. **Sara Zielinska:** Methodology, Software, Writing –

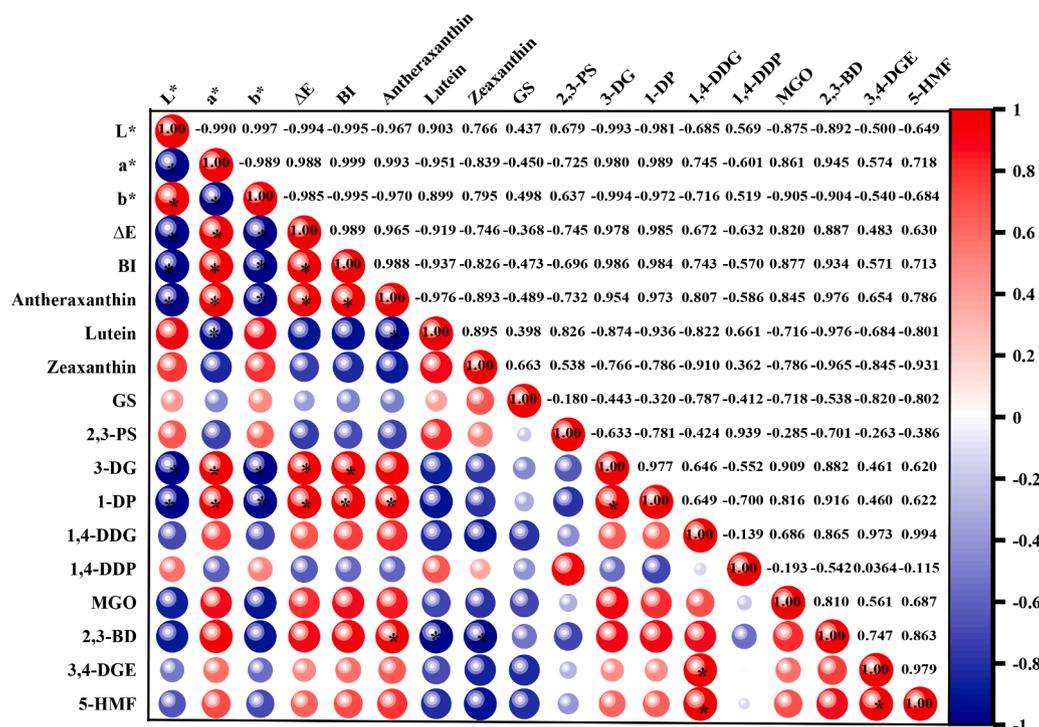


Fig. 6. The correlations between color, α -DCs, 5-HMF and carotenoids of rape bee pollen under different HAD conditions. * means values with superscripts are significantly different, $P < 0.05$.

review & editing. **Jia-Bao Ni**: Writing – review & editing. **Xiang-Xin Li**: Writing – review & editing. **Xiao-Feng Xue**: Formal analysis, Writing – review & editing. **Wen-Li Tian**: Writing – review & editing. **Wen-Jun Peng**: Conceptualization, Funding acquisition, Writing – review & editing. **Xiao-Ming Fang**: Conceptualization, Resources, Writing – review & editing.

Declaration of Competing 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2022.100464>.

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