Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: https://www.sciopen.com/journal/2097-0765

China Food Publishing Co.

Effects of superheated steam treatment on the inactivation of microbial counts, enzyme activity and the inhibition of lipid oxidation of rape bee pollen

Yanxiang Bi[a,1], Zidan Zhou[a,1], Jiabao Ni[a,b], Sara Zielińska[c], Zhihao Zhang[d], Chunliang Luo[e], Wenjun Peng[a], Na Ma[f], Wenli Tian[a,*], Xiaoming Fang[a,*]

[a] State Key Laboratory of Resource Insects, Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing 100093, China
[b] College of Engineering, China Agricultural University, Beijing 100083, China
[c] Department of Systems Engineering, University of Warmia and Mazury in Olsztyn, Olsztyn 10-719, Poland
[d] Mudanjiang Branch of Heilongjiang Academy of Agricltural Sciences, Harbin 157020, China
[e] Yunnan Nongken Nujiang Bee Industry Co., Ltd., Peng Wenjun Expert Workstation, Lushui 673100, China
[f] Zhonghong Bioengineering Co., Ltd., Beijing 100020, China

ARTICLE INFO

Article history:
Received 28 September 2024
Received in revised form 23 October 2024
Accepted 13 November 2024

Keywords:
Rape bee pollen
Superheated steam
Cobalt-60 isotope radiation
Microbial counts
Enzymatic activity
Lipid oxidation

ABSTRACT

The effect of superheated steam (SHS) treatment on the quality characteristics of rape bee pollen were studied, and the efficiency of inactivation and inhibition of lipid oxidation were analyzed to investigate the differences between SHS and cobalt-60 isotope (^{60}Co) radiation treatment. The number of total plate count (TPC) and mold colonies (MC) remained within the limits of the standards after SHS treatment at 140 °C for 2 min. Neither TPC nor MC were detected after ^{60}Co irradiation. Peroxidase (POD) and polyphenol oxidase (PPO) activities significantly decreased with increasing temperature and duration of SHS, while ^{60}Co radiation completely inactivated PPO. Compared to ^{60}Co radiation, SHS treatment inhibited the deterioration of rape bee pollen by avoiding hydroperoxide production and lipid oxidation due to lack of oxygen. These results suggested SHS under 140 °C for 2 min was the most suitable to inactivate the microorganisms and enzymes in rape bee pollen with minimal lipid oxidation.

1. Introduction

Biologically active substances derived from natural sources consistently focus a great interest. Bee pollen is often regarded as "the world's best food product" due to its abundant nutrients, including protein, amino acids, carbohydrates, lipids, phenolic compounds, and vitamins[1]. It is also highly appreciated among consumers for its biological activities such as antioxidant, anti-inflammatory, antimicrobial and anti-atherosclerosis[2]. China is the largest producer of bee pollen worldwide, with an annual output of approximately 5 000 t, accounting for 1/3 of the global production[3]. Currently, bee pollen has been extensively applied in functional foods, beverages, pharmaceuticals, cosmetics, animal feed, and various other domains. However, fresh bee pollen contains 21%–30% (wet basis) water, which promotes the proliferation of microorganisms and even leads to the production of mycotoxins and the activation of enzymes in bee pollen[4]. This reduces the quality of bee pollen and poses potential health risks to consumers. Effective sterilization and inhibition of enzymatic activity are the top priorities to increase the shelf life of bee pollen on the market.

[1] These authors contributed equally to this work.
[*] Corresponding authors at: State Key Laboratory of Resource Insects, Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing 100093, China.
E-mail address: tianwenli@caas.cn (W.L. Tian), fangxiaoming@caas.cn (X.M. Fang)
Peer review under responsibility of Beijing Academy of Food Sciences.

SciOpen Publishing services by Tsinghua University Press

http://doi.org/10.26599/FSHW.2024.9250414

Cobalt-60 isotope (^{60}Co) irradiation is a commonly used commercial sterilization and preservation technique to prolong the shelf life of bee pollen products. An irradiation dose of 10 kGy or lower is generally considered safe by the World Health Organization[5]. However, it may lead to undesirable effects, such as the production of trans fatty acids and a specific "irradiated odor". To ensure food safety more effectively, the sterilization of bee pollen often requires higher doses of ^{60}Co radiation, which highlights the inherent trade-off between sterilization effectiveness and potential impact on product properties and consumer acceptance. Meanwhile, the procession of large quantities of materials with ^{60}Co radiation can also lead to high cost of radiation source and protection device. The application of ^{60}Co radiation technology still encounters limited consumer acceptance due to a lack of awareness and trust in the safety of radiation food products. These challenges highlight the need for research into effective and economical alternative sterilization methods with less impact on the sensory quality to support the development of the bee pollen industry.

Superheated steam (SHS) is an innovative thermal treatment that involves heating saturated steam above its saturation point, resulting in dry saturated steam that is further heated to a temperature above the boiling point. It has a wide temperature range spanning from 100 to 1 000 ℃, encompassing pressure conditions including low, atmospheric, and high pressures. Steam can be generated by electricity, and it operates under non-oxidizing conditions, which prevents or mitigates the combustion reactions[6]. Therefore, this technology exhibits a high heat transfer coefficient, low energy consumption, and exceptional safety[7]. Compared to the conventional heating processing methods, SHS has been successfully applied to food processing for several food commodities to improve the physicochemical properties, nutritional value, and safety of food products[6,8]. Previous studies have shown that the SHS treatment can effectively reduce the population of bacteria and molds, as well as inactivate peroxidase (POD) and polyphenol oxidase (PPO) to improve storage quality[9-11]. Generally, the efficacy of microbial and enzyme inactivation increases with the increase in steam temperature and duration[12]. The utilization of SHS is also beneficial for the products that require the removal of potential spoilage organisms and subsequent dehydration treatment[13]. Both phenomena occur simultaneously, making SHS processing an ideal choice for bee pollen. Additionally, elevated temperatures exert a crucial influence on the stability of volatile compounds in rape bee pollen. For example, high temperature affects changes in fatty acids, molecules of sulfur-containing compounds, the Maillard reaction, and thermal oxidation of lipids. All of these changes can significantly affect the desirable organoleptic properties of food. Therefore, examining the effect of SHS on lipid oxidation may contribute to the selection of optimal conditions for bee pollen treatment.

In this paper, the effect of SHS treatment on the quality properties and sensory attributes of rape bee pollen was studied. A comparison of the effectiveness of the emerging treatment and commercial technology on the inactivation of microorganisms and enzymes as well as lipid oxidation quality in rape bee pollen was highlighted. The aim is to find an appropriate alternative method that can enhance the safety and quality of rape bee pollen while minimizing lipid oxidation. This paper is meaningful in consideration of the potential practicability of SHS treatment for its anaerobic environment and high heat treatment efficiency in food industry, which may provide an effective reference for the processing of bee pollen.

2. Materials and methods

2.1 Materials

Fresh rape bee pollen samples were purchased in March 2022, from Gangzhou Village, Wuhan, China (30° 31′N, 113° 90′E). The initial moisture content was (19.17 ± 0.16)% on wet basis. All the samples were hermetically sealed transported by cold chain (2 – 8 ℃). The whole process of collection, transportation and transfer to the laboratory (Chinese Academy of Agricultural Sciences, Beijing, China) did not exceed 20 h. Samples with consistent size were carefully selected to ensure the uniformity of the material's physicochemical properties. The samples were stored at a temperature of (−18 ± 1) ℃ prior to conducting subsequent experiments.

2.2 Radiation treatment process

The radiation treatment procedure was performed in the laboratory in Beijing Hongyi Sifang Radiation Technology Co., Ltd. (China). Each sterilely packed samples of fresh rape bee pollen weighing (50 ± 5) g were subjected to gamma irradiation using the ^{60}Co as the radiation source at doses of 6, 8 and 10 kGy respectively in BFT-II facilities (MDS-Nordion, Canada). The absorbed radiation dose was measured using polymethyl methacrylate dosimetry system (Harwell, UK). All the samples treated were hermetically sealed and stored at temperature of −18 ℃ before the experiment.

2.3 SHS treatment process

The SHS treatment equipment was developed in the Laboratory of Cereal Science, China Agricultural University, Beijing, China. The equipment was mainly composed of an auto electric heating steam generator, steam distributing system, condenser system, thermal insulation chamber, conveyor belt and monitoring system (SHS temperature and pressure humidity, main control unit) (Fig. 1). Saturated steam is generated by an auto electric heating steam generator, which is heated to the required temperature as superheated steam by adjusting the control panel. The superheated steam is transported through a steam transmission pipe to a steam distributing system located at the top and bottom of the conveyor belt. The superheated steam is injected into the sample trays on the conveyor belt through small holes in the steam pipes. The sample processing time was controlled by the conveyor speed and stagnation.

A single layer of fresh rape bee pollen samples with an average weight of (150 ± 5) g was distributed evenly on the feeder tray (200 mm × 150 mm × 10 mm). The temperature of SHS was conducted at 140 and 170 ℃, respectively, with the processing time varying from 2−6 min in intervals of 2 min at each temperature. After processing, the samples were transferred to a sterilization container and cooled to room temperature. Then, were placed in a sealed sterile bag (114 mm × 229 mm, Hunan BKMAM Biotechnology Co., Ltd., China) and stored at −18 ℃ to ensure sample stability until further analysis.

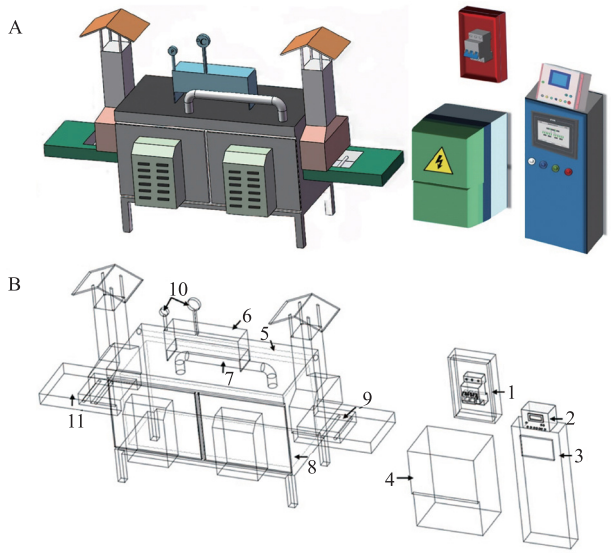


Fig.1 (A) 3D model and (B) schematic diagram of SHS treatment equipment. 1. Air switch; 2. Intelligent four-loop measurement and control instrument; 3. Control panel; 4. Auto electric heating steam generator; 5. Steam transmission pipe; 6. Steam distributing system; 7. Condenser system; 8. Thermal insulation chamber; 9. Conveyor belt; 10. Temperature and pressure inspection instrument; 11. Superheated steam equipment frame.

2.4 Microbiological analysis

The assessment of the microbiological quality of rape bee pollen was carried out following the method describedin the previous literature with some modifications[14]. A sample dilution was performed by weighing (10.00 ± 0.05) g of ground rape bee pollen and dissolving in 90 mL of sterile saline solution. In order to obtain appropriate dilutions, dissolving 1 mL prepared solution in 9 mL sterile saline solution, making as many successive dilutions as necessary. Incubation of plates with total colony-forming units was performed at 37 ℃ for 48 h, while plates with molds were incubated at 28 ℃ for 5 days. The plate-counting method was applied to determine the colony-forming units of bacteria and molds.

2.5 Determination of enzyme activity

2.5.1 POD activity

POD activity was measured as previously reported with some modification[15]. The enzyme extracting solution was prepared by homogenizing (5.00 ± 0.05) g of fresh rape bee pollen sample with 5 mL of an extraction buffer (0.34% polyethylene glycol 6 000 and 4% polyvinylpyrrolidone were weighed and mixed with 1% Triton X-100, then added 0.1 mol/L, pH 5.5 acetic acid-sodium acetate buffer and stirred until dissolved). After centrifugation at 4 ℃ and 12 000 × *g* for 30 min, the supernatant was collected as the enzyme extracting solution. Enzyme activity was measured using a ultraviolet and visible spectrophotometry (UV-2550, SHIMADZU Co., Kyoto, Japan) by rapidly mixing 3 mL guaiacol solution (25 mmoL/L) with 0.5 mL enzyme extracting solution and 200 μL hydrogen peroxide solution in a cuvette within 15 s. Absorbance at wavelength of 470 nm was recorded every minute for no less than 6 points over 3 repetitions to determine 1 unit of enzyme activity. The POD activity was calculated according to the following formula (1):

$$\text{POD activity (U/(g·min))} = \frac{\Delta A_{470\,\text{nm}} \times V_t}{W \times V \times 0.01 \times \Delta t} \quad (1)$$

Where $\Delta A_{470\,\text{nm}}$ indicates the absorbance difference between 1 and 6 min; V_t indicates total volume of sample extract (mL); W indicates the quality of samples (g, dry basis); V indicates volume of sample extract added to the solution (mL); Δt indicates the reaction time in linear area (min).

2.5.2 PPO activity

PPO activity was measured according to previously reported literature[16]. The preparation of enzyme extracting solution was the same as that described in the Section 2.5.1. The volume of 4.0 mL of acetic acid-sodium acetate buffer (50 mmol/L, pH 5.5), 1.0 mL of pyrocatechol (50 mmol/L), and 100 μL of the enzyme extracting solution were mixed rapidly in a 10 mL centrifuge tube and then loaded it in a cuvette within 15 s for measurement. Change in absorbance was measured at 420 nm. The PPO activity was calculated according to the following formula (2):

$$\text{PPO activity (U/(g·min))} = \frac{\Delta A_{420\,\text{nm}} \times V_T}{W_D \times V_S \times 0.01 \times \Delta t} \quad (2)$$

Where $\Delta A_{420\,\text{nm}}$ indicates the absorbance difference between 1 and 6 min; V_T indicates total volume of sample extract (mL); W_D indicates the quality of samples (g, dry basis); V_S indicates volume of sample extract added to the test solution (mL); Δt indicates the reaction time in linear area (min).

The enzyme inactivation rate (EIR) for both the analyzed enzymes (POD and PPO) was calculated using the following formula (3):

$$\text{EIR}\,(\%) = \frac{A_0 - A}{A_0} \times 100 \quad (3)$$

Where A_0 indicates the enzyme activity of the untreated bee pollen, and A indicates the enzyme activity of bee pollen after different SHS treatment time.

2.6 Measurement of color parameters

The color parameters of the rape bee pollen samples were measured using a CM-5 spectrophotometer (Konica Minolta, Japan). The system provides the values of 3 color components; L^* (black, white), a^* (+ red, – green) and b^* (+ yellow, – blue). Based on the L^*, a^*, b^* parameters, the total color difference (ΔE) between the fresh and treated samples was calculated according to the formula (4):

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (4)$$

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

The browning index (BI) was calculated using the following formulas (5) and (6)[17]:

$$\text{BI} = \frac{100 \times (X - 0.31)}{0.17} \quad (5)$$

$$X = \frac{(a^* + 1.75L^*)a^*}{5.645L^* + a^* - 3.012b^*} \quad (6)$$

Where L^*, a^*, b^* indicate the lightness, redness, and yellowness of treated rape bee pollen.

Each sample was tested 6 times and the average value was taken.

2.7 Microstructure observation

The conductive adhesive was respectively evenly coated with an appropriate amount of fresh sample and samples treated with SHS and irradiation successively. Subsequently, a thin layer of gold was sputtered onto the samples. Morphological analyses of the samples were performed using a scanning electron microscope (SEM) model SU3500 (Hitachi, Japan) at an accelerating voltage of 10.0 kV.

2.8 Determination of malondialdehyde (MDA)

MDA content in the samples was determined using a testing kit (Beijing Solabo Biotechnology Co., Ltd., China) stored in a refrigerator at –4 ℃. Samples weighing 0.1 g were homogenized and extracted in 5 mL phosphate buffer saline (PBS) with a concentration of 0.05 mol/L and pH 7.8 at 4 ℃. The supernatant obtained from the sample and the control were transferred into a screw-capped tube, and 2.5 mL thiobarbituric acid was added. Then the tubes were placed in a boiling water bath for 60 min and quickly cooled in an ice bath. After centrifuging at 10 000 × *g* for 10 min, the absorbance of the supernatant was measured at 532 and 600 nm, respectively.

$$\Delta A = \left(\Delta A_{532D} - \Delta A_{532B}\right) - \left(\Delta A_{600D} - \Delta A_{600B}\right) \quad (7)$$

Where ΔA_{532D} and ΔA_{600D} indicate the absorbance values at 532 and 600 nm for each sample in the treatment group; ΔA_{532B} and ΔA_{600B} indicate the absorbance values at 532 and 600 nm for the blank group.

2.9 Extraction and determination of fatty acids

Fatty acids were determined using the method outlined in the previous literature with slight modifications[18]. Crude lipids were extracted from the sample with a solvent of petroleum ether. The mass of (20 ± 1) g of pollen was ground and mixed with 40 mL of NaCl solution. After adding 250 mL of petroleum ether and centrifuging for 10 min (10 000 × *g*). The supernatant was washed by deionized water in the ratio of 1:1, then the organic phase was collected and freeze-dried after nitrogen blowing. The concentration of 0.4 mol/L NaOH-MEOH (10 mL) and 5% H_2SO_4-MEOH solution were added respectively to crude lipids ((50.00 ± 0.05) mg) and mixed thoroughly, then placed in a water bath at 70 ℃ for 1 h. After cooling to room temperature, the volume of 5 mL of 5% NaCl solution and 10 mL of hexane were added. After repeating the extraction 3 times, the hexane phase was collected and then dried with nitrogen gas for further analysis.

The fatty acids were subsequently analyzed with a GPC-GC/MS 2010 plus (Shimadzu, Kyoto, Japan) coupled with a capillary column DB-FastFAME (30 m × 0.25 mm, 0.25 μm). Chromatographic analysis was performed at a constant nitrogen flow rate (purity 99.99%) of 1.0 mL/min. A volume of 1 μL prepared sample was measured and injected using an injector temperature of 250 ℃ in 1:50 split mode. The parameters of the chromatographic column were set as follows: initial temperature at 70 ℃ held for 3 min; then increased to 195 ℃ at 35 ℃/min and held for 3 min; after which the temperature was raised to 235 ℃ at a rate of 10 ℃/min and held for 6 min. The total duration of analysis was 26 min. The start and end of the ratio were *m*/*z* 40 and 500, respectively, with the mass detector and interface temperature being 250 ℃.

2.10 Determination of volatile compounds

The mass of (1.00 ± 0.05) g of fresh and treated rape bee pollen was ground into powder and placed in a headspace extraction bottle. After that, a volume of 5 μL of 2-nonanone (500 mg/L) was added as an internal standard solution, and the mixture was fully vortexed after sealing. The volatile metabolites were collected with the solid phase micro extraction (SPME). The sample equilibration process was carried out at 45 ℃ for 15 min, and SPME head (model: 50/30 μm DVB/CAR/PDMS) was then inserted into the sample bottle for 45 min to enrich the volatile metabolites. Finally, the SPME head was transferred into the GC-MS sample inlet for desorption.

Volatile metabolites of fresh and treated rape bee pollen were measured by the Agilent 5975C-7890A gas chromatography-mass spectrometer (Agilent, USA). INNOWAX capillary chromatographic column (30 m × 0.25 mm, 0.25 μm, Agilent, USA) was used to separate the volatiles with the carrier gas flow rate at 1.0 mL/min. The separated volatiles were ionized at 70 eV electron energy with the ion source temperature at 250 ℃. The temperature rise procedure was started at a temperature of 40 ℃, then the temperature was increased to 160 ℃ at a rate of 2 ℃/min, and then to 240 ℃ at a rate of 10 ℃/min. Retrieval and comparison are conducted through NIST/EPA/NIH Mass Spectral and Wiley Registry of Mass Spectral Data.

2.11 Statistical analysis

Statistical analysis of the data was performed using SPSS 26.0 (SPSS Inc., Chicago, IL, USA) and Origin Pro 2021 (OriginLab Inc., Northampton, MA, USA) software. All analyses were performed in triplicate, and the results were presented as the mean ± standard deviation. One-way analysis of variance (ANOVA) and Duncan's multiple range test at $P < 0.05$ was conducted to determine the statistically significant differences between samples.

3. Results and discussion

3.1 Results of microbiological analysis

Changes in TPC and MC in rape bee pollen under the influence of various SHS treatment and ^{60}Co radiation conditions were investigated (Fig. 2). Microbiological results indicated a serious microbial contamination of fresh rape bee pollen. TPC levels decreased significantly after SHS treatment at 140 and 170 ℃ for the first 2 min, resulting in a reduction of 2.12 and 2.34 (lg (CFU/g)), respectively. The decline then became not statistically significant with increasing treatment duration. The effects of heat on bacterial cells can mainly be attributed to the damage to bacterial proteins and the permeabilization of plasma membrane[19]. When the cell is heated, the water molecule vibrated, which weakens and breaks the disulfide and hydrogen bonds in the proteins surrounding it and deactivates its functionality. On the other hand, the moisture content of rape bee pollen decreased with increasing SHS treatment time, and water loss by bacterial cells led to cell shrinkage and thickening of cell

membrane, which showed a certain protective effect against thermal damage[20]. This phenomenon also indicated the possibility of persistence of heat-resistant sub populations of microorganisms in rape bee pollen. Similar trends were found in the case of microbiological disinfection of wheat grain with SHS treatment[19].

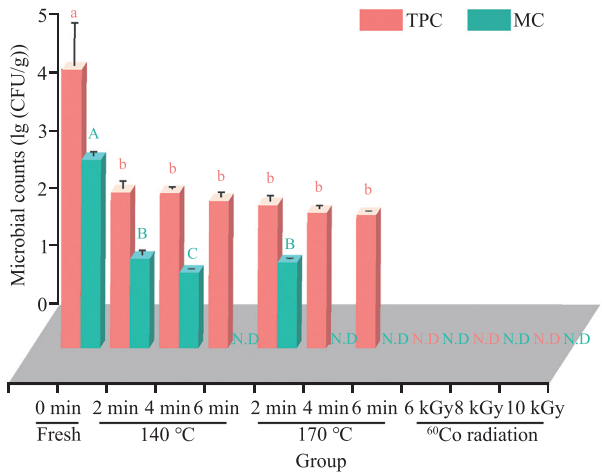


Fig. 2 Changes in the number of TPC and MC in rape bee pollen treated with different SHS conditions and ^{60}Co radiation. N.D, not detected. Different lowercase letters indicate significant difference $P < 0.05$. Different uppercase letters indicate significant difference $P < 0.05$.

After 2 min of SHS treatment at 140 and 170 °C, approximately 1.72 and 1.78 (lg (CFU/g)) MC were inactivated in rape bee pollen, respectively. MC cannot be detected after SHS treatment at 140 °C for 6 min and 170 °C for 4 and 6 min. Compared to TPC, MC were relatively easy to eliminate. The results indicate that moist heat can trigger spore germination, weakening the heat resistance of microorganisms. A similar trend was found in the case of SHS steam sterilization of highland barley[21].

In samples irradiated with 6, 8 and 10 kGy of ^{60}Co, no TPC and MC populations were detected. This is because radiation can cause direct damage several components of the cell, including the genetic material destroy microorganisms[22]. This not only randomly disrupts cellular functions, but also deprives microorganisms of the ability to replicate or regenerate. In addition, other cell components were exposed to ^{60}Co radiation interactions, even without disruption of genetic materials, the damaging effects on cellular proteins and cell membranes can reduce the chances of survival of damaged cells[23].

For rape bee pollen, the TPC reduction to less than 4.00 (lg (CFU/g)) and the MC reduction to less than 2.30 (lg (CFU/g)) achieve the acceptable microbiological standard of ready-to-eat pre-packaged bee pollen products in National Standards of People's Republic of China (2016)[24]. It was observed that SHS treatment for 2, 4, 6 min at 140 and 170 °C and ^{60}Co radiation treatment significantly reduces the microbial load to reach the safe levels for rape bee pollen. The results indicated that ^{60}Co radiation is more efficient than SHS treatment in reducing the microbial load of rape bee pollen.

3.2 *Enzyme inactivation*

POD and PPO are key enzymes affecting food quality and shelf life. The effect of SHS treatment on the relative POD and PPO activities of rape bee pollen is shown in Fig. 3. It can be observed that POD and PPO were inactivated rapidly within the first 2 min of SHS treatment. An increase in SHS temperature and treatment time increased the inactivation rate of the 2 enzymes. The relative activity of POD decreased to 43.10% after 6 min at 140 °C and to 39.02% at 170 °C for 2 min. The relative activity of PPO decreased to 4.08% and 3.57% at 140 and 170 °C for 6 min, respectively. Exposure of POD and PPO to SHS denatured proteins and affected catalytic function, thereby causing a decrease in enzymes activities. Meanwhile, these facts indicate that the resistance of POD against the heat treatment may be higher than that of PPO. Some studies found similar results that the inactivation trend of PPO was similar to that of POD, but PPO was more temperature sensitive and had a higher degree of inactivation[25]. It was reported that the residue activities of POD and PPO in apricot were extensively reduced with increasing of the blanching temperature and time[26]. A study reported that heat-treated of strawberry puree after 90 °C for 15 min result in 97.7% and 99.5% enzyme inactivation of PPO and POD, respectively[27]. Overall, these studies demonstrated that SHS is a highly effective inactivation technology suitable for commercial application.

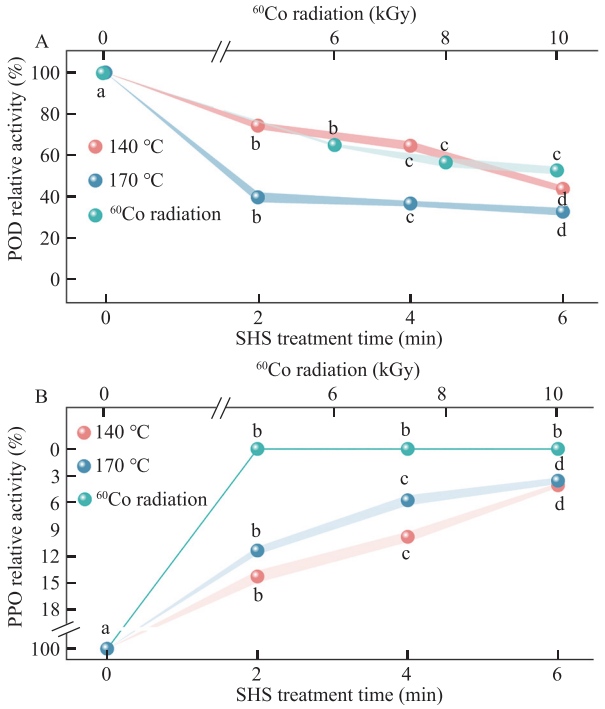


Fig. 3 Effect of SHS and ^{60}Co radiation on (A) POD and (B) PPO relative activities of rape bee pollen. Different lowercase letters in the same line indicate significant difference $P < 0.05$.

The POD relative activities gradually decreased with the increasing absorbed dose and no PPO activity was detectable after ^{60}Co radiation. The inhibitory effect of POD was greater with ^{60}Co radiation at 6 and 8 kGy compared to SHS treatment at 140 °C for 2 and 4 min. However, when the ^{60}Co radiation dosage increased to 10 kGy, the inhibitory effect of POD was lower than that observed with SHS treatment. It seems that ^{60}Co radiation was more effective in inactivating of PPO activity. The same mild and continuous pattern of inactivation process of POD activity was also described by Constantinovici et al.[28]. The mechanism of radiation inactivation

may occur through a transitional conformational state that leads to structural disruption of the enzyme with increasing doses of radiation. The incident ions damage the surface tissue of rape bee pollen, and then the energy transfer from radiation causes random degradation of some amino acid residues, ultimately leading to structural degradation of the enzyme[29].

3.3 Color analysis results

The color of rape bee pollen is a key indicator of its acceptance by consumers, reflecting the organoleptic properties of the product. The effects of different SHS treatment and ^{60}Co radiation conditions on the rape bee pollen surface color parameters, such as L^*, a^*, b^*, ΔE and BI of are shown in Table 1. It was found that the L^* of SHS treated rape bee pollen decreased significantly from 54.83 ± 0.27 to 51.44 ± 0.21 ($P < 0.05$). High temperature resulted in a decrease in L^* values for all treatments as the SHS treatment time increased. The a^* values of SHS groups were higher than that of the fresh sample, while the b^* value was significantly decreased ($P < 0.05$) after SHS treatment. The results show that SHS treatment significantly reduces the L^* values and modifies other color parameters, which indicates changes in the visual perception of pollen. The prolonged exposure to SHS leads to the inactivation of oxidative enzyme such as POD and PPO, responsible for deterioration reactions that contribute to undesirable color and accompanied by the Maillard reaction caused by high temperatures[30]. This is consistent with the results obtained regarding the rate of enzyme inhibition. The Maillard reaction occurring at high temperatures was a key factor leading to the formation of a brown nitrogen-containing polymer, turning the samples into a deeper dark brown and red color. Most probably, the destruction of cell structure caused by SHS facilitated the release of carotenoids from the matrix into the intercellular space, which may make the rape bee pollen redder and yellower. A study reported that carrots subjected to vacuum-steam pulsed blanching were redder than carrots blanched in hot water, which may be related to the low nutrient loss by diffusion or leaching during the blanching process as it was used steam as the thermal medium[30]. The change in the b^* value may also be caused by oxidation reactions of some thermosensitive compounds during SHS treatment. During the SHS treatment process, the increase in temperature facilitated the mobility of fat and flavonoids molecules and enhance the oxidation reactions. A previous report studied the SHS treatment of durian chips and confirmed lower L^* values, and higher a^*, b^* values in samples exposed to elevated[31]. This finding is consistent with our experimental results.

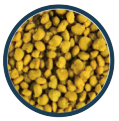
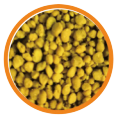
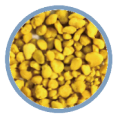
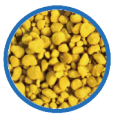
Table 1 shows that the values of ΔE index of rape bee pollen increased from 5.58 ± 0.66 to 12.85 ± 0.68 with the increase in SHS time at 140 and 170 °C. In addition to pigment degradation and Maillard reaction, the increase in ΔE values may also be the result of high blanching temperature on heat-sensitive components, such as proteins and carbohydrates amongst others[32]. In this study, BI increased with increasing SHS treatment time and temperature. The rate of change in BI of samples subjected to SHS at 170 °C was faster than that at 140 °C. It has been reported that the higher temperature increases the mobility of amino acid reducing sugar molecules, resulting in the acceleration of Maillard reaction rate[33].

Compared to fresh samples, the L^* and b^* values of pollen gradually decreased with increasing radiation dose. However, they were still higher than that subjected to SHS treatment. ^{60}Co radiation increased the ΔE value from 2.32 ± 0.07 to 5.44 ± 0.04. Browning was greater after SHS treatment due to ΔE was higher (from 5.58 ± 0.66 to 9.44 ± 0.56 at 140 °C and 8.43 ± 0.49 to 12.85 ± 0.68 at 170 °C). As ^{60}Co radiation completely inactivated the of activities of TPC, MC and POD, which were responsible for the browning of food, it can reduce the degree of oxidation of phenolic compounds to brown melanin, thereby improving the color parameters.

3.4 Microstructure

Analysis of the microstructure of rape bee pollen carried out using SEM revealed morphological diversity depending on the processing conditions (Fig. 4). Fresh rape bee pollen showed a spherical shaped with loosely scattered granules and a dense, net-like surface of the outer wall. It was well arranged with germinal apertures, and 3 germinal furrows can be observed on the cell surface. The furrows were wide in the middle and narrow at both ends. Thermal and radiation treatment introduced changes such as cracks in cell walls, deformations and loss of structure, which indicates the impact of these processes on the cellular integrity of pollen. The outer wall of rape bee pollen cells began to rupture from the germinal aperture below 140 °C. As the SHS time increased, the pollen became slightly distorted and the width of the embryonic opening increased. The structure of rape bee pollen cell wall was damaged and several fragments were decomposed. It was found that the cracks in the germinal aperture gradually enlarged and deepened at 170 °C. The shape of the cell

Table 1
Color characteristics of rape bee pollen under different SHS treatment and ^{60}Co radiation conditions.

Index	Fresh	140 °C			170 °C			^{60}Co radiation		
		2 min	4 min	6 min	2 min	4 min	6 min	6 kGy	8 kGy	10 kGy
										
L^*	67.45 ± 0.35^{a}	54.83 ± 0.27^{d}	52.51 ± 0.19^{e}	52.21 ± 0.19^{e}	51.87 ± 0.32^{ef}	51.86 ± 0.27^{ef}	51.44 ± 0.21^{g}	65.91 ± 0.06^{b}	64.80 ± 0.06^{c}	64.50 ± 0.02^{c}
a^*	3.48 ± 0.13^{g}	6.17 ± 0.13^{d}	8.39 ± 0.13^{c}	9.20 ± 0.14^{b}	5.70 ± 0.06^{e}	9.24 ± 0.14^{b}	12.60 ± 0.14^{a}	5.00 ± 0.01^{f}	5.20 ± 0.03^{f}	5.22 ± 0.01^{f}
b^*	58.82 ± 0.20^{a}	47.95 ± 0.44^{d}	47.52 ± 1.07^{d}	48.55 ± 0.27^{d}	45.82 ± 0.08^{e}	48.45 ± 0.34^{d}	51.71 ± 0.40^{c}	57.96 ± 0.09^{a}	55.93 ± 0.14^{b}	54.59 ± 0.04^{b}
ΔE		5.58 ± 0.66^{c}	8.80 ± 0.39^{b}	9.44 ± 0.56^{b}	8.43 ± 0.49^{b}	9.72 ± 0.46^{b}	12.85 ± 0.68^{a}	2.32 ± 0.07^{d}	4.28 ± 0.12^{c}	5.44 ± 0.04^{c}
BI	1 018.83 ± 36.16^{f}	1 982.80 ± 60.63^{d}	2 880.04 ± 62.80^{c}	3 271.04 ± 57.05^{b}	1 832.63 ± 21.49^{d}	3 298.84 ± 56.72^{b}	4 941.53 ± 67.36^{a}	1 564.23 ± 1.89^{e}	1 607.75 ± 11.34^{e}	1 588.56 ± 3.63^{e}

Note: Data are shown as the mean ± standard deviation ($n = 3$). Different lowercase letters in the same row mean significantly different ($P < 0.05$).

became irregular and the inner wall of the cell collapsed inward at 170 °C for 4 min. When the treatment time was further increased to 6 min, the outer wall of rape bee pollen cells detached from the surface and the inner cell wall collapsed severely.

Analysis of the microstructure of rape bee pollen revealed that the germinal apertures of rape bee pollen are located at the opening of the thin-walled area of the outer cell wall that cannot be protected by the thick and hard outer wall of the cell, that is, the position of germinal apertures is characterized by low mechanical strength. Exposure to SHS, they were easily dehisced from the germinal apertures, causing damage and breakdown of the cell tissue. This process promotes the expansion of micro-channels and rupture of the cell walls, which may result in the release of cell contents. Several studies have reported that the mechanical breakdown of the pollen layer is beneficial to increase the digestibility and consequently bioavailability of bee pollen grains[3]. Additionally, the effect of SHS can cause the cell wall broke into several fragments and fall off, making cells more susceptible to deformation.

Microstructural analysis of rape bee pollen after ^{60}Co radiation at 6 kGy showed a morphological transformation from a spherical to an ovel form, characterized by a shallow and dense reticulated surface. It gradually became distorted and irregular in shape with increasing radiation dose. This phenomenon was correlated with changes in the microcrystalline structure of cellulose and pectin solubilization, highlighting the sensitivity of internal components such as cellulose and pectin to radiation[34]. It was found that the outer membrane of bee pollen is made up of sporopollenin and the intine layer consists of cellulose and pectin with a more sensitive structure compared to the outer layer. ^{60}Co radiation used an applied electromagnetic field to easily disrupt the microstructure of bee pollen by disordering the cellulose molecular arrangement[35].

3.5 Free fatty acids

Free fatty acids are typically produced by the lipolysis of phospholipids and triacylglycerols, acting as precursors for volatile flavor compounds such as aldehydes, alcohols, and ketones that are generated by oxidation[36]. The levels of these free fatty acids are the result of the processes of lipid hydrolysis and oxidation, as they serve as intermediate products in lipid oxidation. Free fatty acid compositions of fresh bee pollen, SHS treated and ^{60}Co radiation treated ones were listed in Table 2. The dominant fatty acids in the examined rape bee pollen were linolenic acid, ($C_{18:3n3}$), palmitic acid ($C_{16:0}$) and myristic acid ($C_{14:0}$), which constituted 70.93% in the total fatty acids. Both saturated and unsaturated fatty acids were decomposed by heat or radiation through the nonoxidative or oxidative reactions to form volatile fatty acids, aldehydes, ketones. Changes in minor fatty acids were may result from the thermal decomposition of monoacylglycerols, diacylglycerols, and triacylglycerols led to the accumulation of those fatty acids in the samples[37].

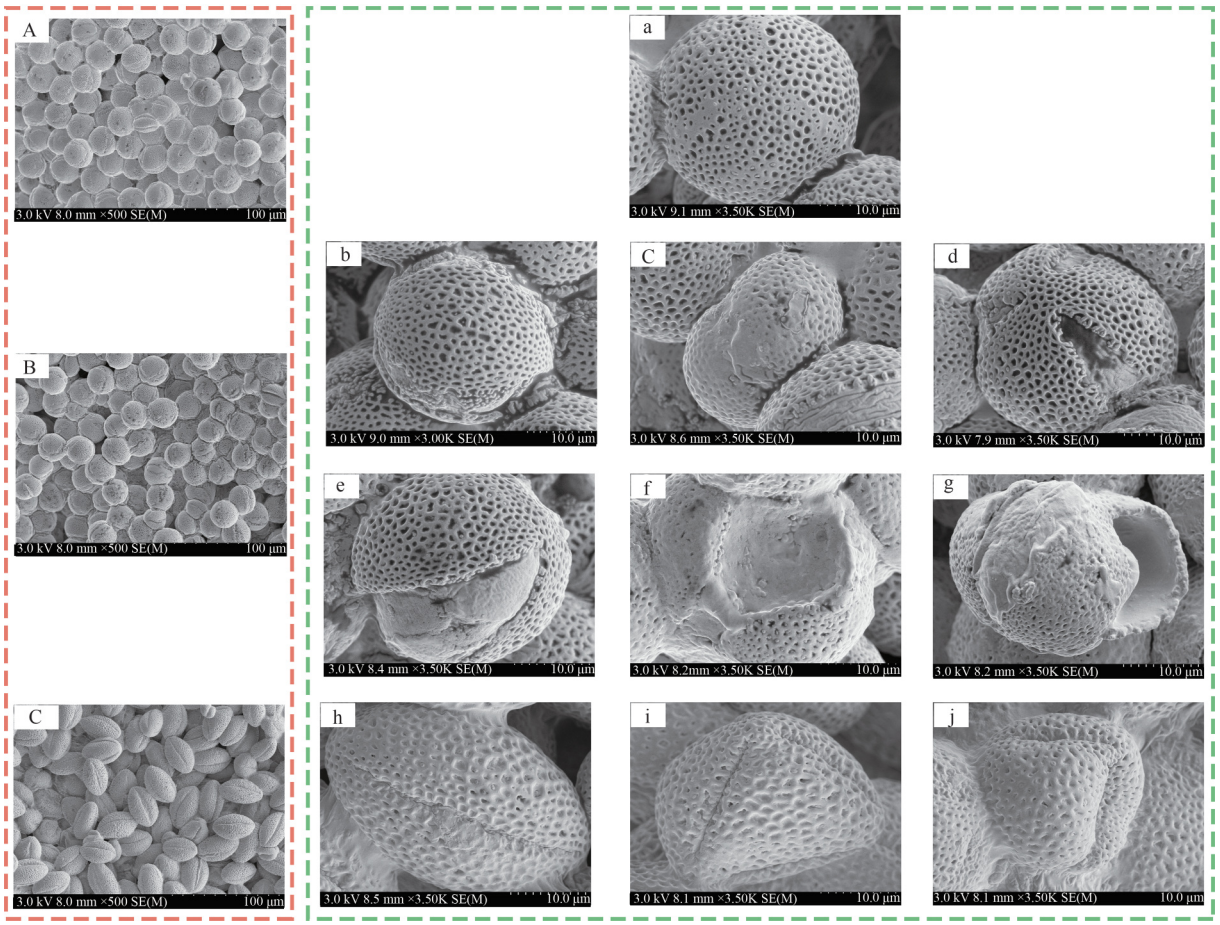


Fig. 4 SEM of rape bee pollen treated by different treatment conditions. (A) Fresh rape bee pollen; (B) 140 °C, 2 min; (C) 6 kGy; (a) fresh rape bee pollen and SHS treated at the temperature and time of (b) 140 °C, 2 min; (c) 140 °C, 4 min; (d) 140 °C, 6 min; (e) 170 °C, 2 min; (f) 170 °C, 4 min; (g) 170 °C, 6 min; (h) 6 kGy; (i) 8 kGy; (j) 10 kGy.

Table 2
Fatty acid compositions and concentrations of rape bee pollen under different treatments (mg/kg).

Fatty acids	Fresh	140 °C			170 °C			^{60}Co radiation		
		2 min	4 min	6 min	2 min	4 min	6 min	6 kGy	8 kGy	10 kGy
$C_{6:0}$	335.08 ± 8.62^{b}	210.00 ± 6.25^{c}	652.54 ± 0.81^{a}	88.80 ± 3.16^{e}	34.48 ± 2.33^{f}	91.72 ± 1.01^{e}	46.23 ± 2.58^{f}	43.14 ± 1.65^{f}	43.02 ± 1.42^{f}	185.23 ± 10.07^{d}
$C_{8:0}$	50.77 ± 1.05^{b}	41.04 ± 2.34^{c}	60.15 ± 1.95^{a}	22.36 ± 0.93^{e}	14.36 ± 1.04^{g}	30.48 ± 0.27^{d}	19.02 ± 1.26^{f}	10.84 ± 0.34^{h}	10.89 ± 0.26^{h}	27.64 ± 0.78^{d}
$C_{10:0}$	81.71 ± 1.88^{a}	56.52 ± 0.43^{c}	66.38 ± 0.51^{b}	39.01 ± 1.61^{f}	29.75 ± 0.10^{h}	48.04 ± 1.90^{d}	32.60 ± 0.31^{g}	22.03 ± 0.77^{i}	23.19 ± 0.68^{i}	44.00 ± 0.50^{e}
$C_{12:0}$	531.69 ± 2.64^{a}	352.10 ± 0.10^{e}	398.58 ± 7.50^{b}	326.90 ± 6.16^{d}	240.31 ± 1.97^{f}	314.36 ± 14.87^{d}	245.72 ± 4.57^{f}	142.58 ± 3.89^{g}	154.86 ± 4.26^{g}	283.71 ± 5.38^{e}
$C_{14:0}$	9 386.11 ± 45.39^{a}	7 560.12 ± 98.75^{c}	7 815.32 ± 146.08^{bc}	8 119.74 ± 50.90^{b}	5 232.56 ± 34.16^{f}	6 407.88 ± 444.17^{d}	5 230.92 ± 23.04^{f}	2 857.78 ± 5.09^{g}	3 157.43 ± 23.87^{g}	5 792.51 ± 94.68^{e}
$C_{15:0}$	60.95 ± 0.43^{c}	53.28 ± 1.17^{d}	67.00 ± 1.49^{b}	85.63 ± 1.17^{a}	41.28 ± 0.85^{e}	32.05 ± 1.60^{f}	58.31 ± 3.01^{c}	15.06 ± 1.27^{g}	16.60 ± 0.65^{g}	32.73 ± 0.90^{f}
$C_{16:0}$	18 652.47 ± 136.01^{b}	15 121.62 ± 210.14^{d}	16 787.11 ± 83.68^{c}	20 298.85 ± 21.13^{a}	11 493.33 ± 200.55^{f}	10 733.29 ± 426.95^{g}	13 591.01 ± 128.03^{e}	6 800.59 ± 112.03^{h}	7 235.90 ± 44.60^{h}	14 809.41 ± 19.20^{d}
$C_{16:1}$	64.30 ± 4.74	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
$C_{17:0}$	62.86 ± 1.66^{b}	48.67 ± 2.10^{c}	61.34 ± 1.75^{b}	85.01 ± 2.12^{a}	36.58 ± 0.90^{e}	31.99 ± 0.72^{f}	52.27 ± 0.51^{c}	19.10 ± 1.38^{g}	20.61 ± 0.89^{g}	41.32 ± 3.88^{a}
$C_{18:0}$	8 112.66 ± 119.90^{a}	5 548.55 ± 48.10^{c}	5 635.00 ± 55.86^{c}	6 632.83 ± 95.84^{b}	3 787.14 ± 28.96^{e}	4 378.85 ± 296.38^{d}	3 829.26 ± 9.27^{e}	3 235.54 ± 59.37^{f}	3 472.48 ± 49.13^{f}	6 783.18 ± 186.146^{b}
$C_{18:1cis}$	5 796.27 ± 142.00^{a}	2 406.29 ± 26.93^{c}	2 495.28 ± 12.99^{c}	3 324.63 ± 57.43^{b}	1 518.33 ± 47.16^{e}	1 732.86 ± 76.40^{d}	1 798.29 ± 13.83^{d}	1 424.74 ± 23.79^{e}	1 565.91 ± 3.14^{e}	2 539.26 ± 37.47^{c}
$C_{18:2cis}$	3 852.78 ± 86.07^{a}	2 552.13 ± 114.18^{cd}	2 685.74 ± 175.62^{c}	3 611.38 ± 0.29^{b}	1 809.69 ± 91.87^{e}	1 734.89 ± 148.96^{e}	2 353.35 ± 12.75^{d}	856.79 ± 1.45^{g}	947.57 ± 3.48^{g}	1 446.77 ± 59.41^{f}
$C_{18:3n3}$	23 081.43 ± 247.53^{b}	18 368.44 ± 146.76^{e}	20 651.81 ± 73.08^{c}	24 983.03 ± 558.48^{a}	13 930.99 ± 183.97^{f}	12 810.80 ± 575.30^{g}	19 318.34 ± 183.03^{d}	4 081.19 ± 105.93^{i}	4 718.87 ± 84.41^{i}	8 050.46 ± 105.93^{h}
$C_{20:0}$	1 608.46 ± 5.70^{a}	1 187.64 ± 26.39^{c}	1 214.62 ± 12.04^{c}	1 362.33 ± 15.43^{b}	728.31 ± 20.95^{e}	1 170.26 ± 28.34^{c}	822.87 ± 29.06^{d}	437.08 ± 437.08^{g}	504.54 ± 16.59^{f}	874.72 ± 54.98^{d}
$C_{22:0}$	119.10 ± 6.26	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
$C_{24:1}$	271.09 ± 4.59^{b}	198.98 ± 9.38^{d}	245.91 ± 5.84^{c}	417.85 ± 15.80^{a}	111.25 ± 3.08^{f}	156.97 ± 4.66^{e}	397.36 ± 16.98^{a}	N.D	N.D	N.D
∑SFA	39 001.85 ± 329.54^{a}	30 179.55 ± 395.79^{d}	32 758.03 ± 311.68^{c}	37 061.47 ± 198.47^{b}	21 638.09 ± 291.82^{h}	23 238.93 ± 1 216.20^{g}	23 928.20 ± 202.62^{f}	13 583.73 ± 1 887.17^{j}	14 639.51 ± 142.36^{i}	28 874.45 ± 376.52^{e}
∑MUFA	6 131.66 ± 151.33^{a}	2 605.27 ± 36.31^{d}	2 741.19 ± 18.83^{c}	3 742.48 ± 73.23^{b}	1 629.58 ± 50.24^{h}	1 889.82 ± 81.05^{g}	2 195.64 ± 30.82^{f}	1 424.74 ± 23.79^{j}	1 565.91 ± 3.14^{i}	2 539.26 ± 37.47^{e}
∑PUFA	26 934.21 ± 333.60^{b}	20 920.57 ± 260.94^{e}	23 337.55 ± 248.70^{c}	28 594.41 ± 558.77^{a}	15 740.67 ± 275.84^{f}	14 545.69 ± 724.27^{g}	21 671.69 ± 195.78^{d}	4 937.98 ± 107.38^{j}	5 666.44 ± 87.89^{i}	9 497.22 ± 165.34^{h}

Note: Values are mean ± standard deviation (n = 3). Different lowercase letters in the same row mean significantly (P < 0.05) different. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. N.D, Not detected.

The oxidative deterioration of lipids often occurs when they are heated at elevated temperatures for an extended time in the presence of atmospheric oxygen. The reduction in total free fatty acids levels as flavor precursors after processing could be attributed to increased oxidation[36]. However, during the SHS treatment, the production of hydroperoxides and oxidative rancidity was avoided due to the lack of oxygen, which can inhibit the deterioration of rape bee pollen quality. Meanwhile, the content of fatty acids exhibits a significant decrease when subjected to SHS treatment at 170 ℃, in comparison to the SHS treatment conducted at 140 ℃. This suggests that a strong effect of high temperature on the degradation of lipids. A similar result showed that SHS lead to a reduction in total fatty acids contents and effectively inhibit lipid oxidation in rice during processing[37]. However, due to the short wavelength of gamma rays, lipids oxidation is easier to accelerate and more fatty acids are lost compared to SHS. MUFA and polyunsaturated fatty acids (PUFA) are very susceptible to oxidation due to the presence of high reactive π bonds, thus they are oxidized to a greater extent than saturated fatty acids and lead to the formation of a characteristic aging flavor during postprocessing storage[38]. It has been reported that intracellular reactive oxygen species generated by ultraviolet radiation can produce harmful chemical species capable of lipid peroxidation, such as hydroxyl radicals, superoxide radicals, or their protonated form, or hydrogen peroxide[39]. These chemical oxidants negatively affected PUFAs, which are particularly susceptible to oxidation. Additionally, hydroperoxides are highly unstable compounds that begin to decompose upon reaching a certain concentration in the food system, leading to the formation of alkoxy radicals. These radicals can further react through various pathways to produce hydrocarbons, alcohols, aldehydes, acids, and other compounds, contributing to the characteristic rancid smell of oxidized lipids. Zhang et al.[40] highlighted that PUFAs exhibit high susceptibility to lipid peroxidation triggered by environmental free radicals, resulting in the formation of MDA, a marker of oxidative stress and lipid degradation.

3.6 MDA analysis results

MDA is considered as a key marker of lipid peroxidation and is the symbol for the oxidized flavor (rancid or warmed-over flavor) generating during food storage or treatment. Analysis of MDA levels in rape bee pollen samples, as illustrated in Fig. 5, showed their increase after various treatments. The highest MDA content was observed after treatment at 170 ℃ for 6 min among all treatment groups, which was 164.62% higher than the level in fresh samples. The values for samples irradiated with 6, 8, and 10 kGy were 114.99%, 151.85%, and 155.95%, respectively. Such results can be attributed to the capability of peroxide with α- or, β-unsaturations to the peroxide group to undergo cyclization to form MDA during SHS treatment[41]. Increased temperature accelerated the lipid oxidation process, which resulted in a further increase in MDA levels, suggesting continued peroxide-catalyzed oxidation. Moreover, higher doses of radiation were found to stimulate lipid oxidation in rape bee pollen samples. The results correlate with the observations of Liu et al.[42], who noticed a significant increase in MDA content in peanut seeds with an increase in radiation dose from 5 to 10 kGy.

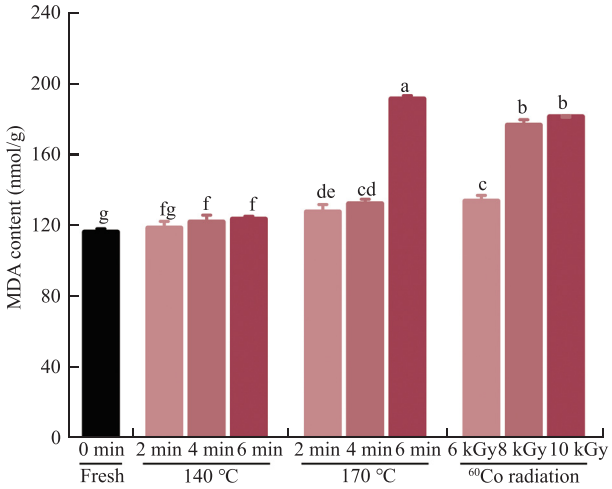


Fig. 5 MDA content of rape bee pollen treated by SHS and ^{60}Co radiation treatment. Different lowercase letters in the same line indicate significant difference ($P < 0.05$).

3.7 Volatile composition

A total of 26 volatile compounds derived from the oxidation of fatty acids was detected in rape bee pollen samples in this study, including acids (7), esters (5), aldehydes (5), ketones (4), alcohols (2), nitrogenous compounds (2), and alkane (1) (Fig. 6, Table 3). These compounds, especially acids, aldehydes and ketones, are quantitatively dominant in treated rape bee pollen, which derived from the oxidation of fatty acids and Maillard reaction. The presence of small amounts of these volatile substances is important for producing the desired aroma of rape bee pollen. However, their excess may contribute to the appearance of undesirable odors and accelerate the rancidity process of products.

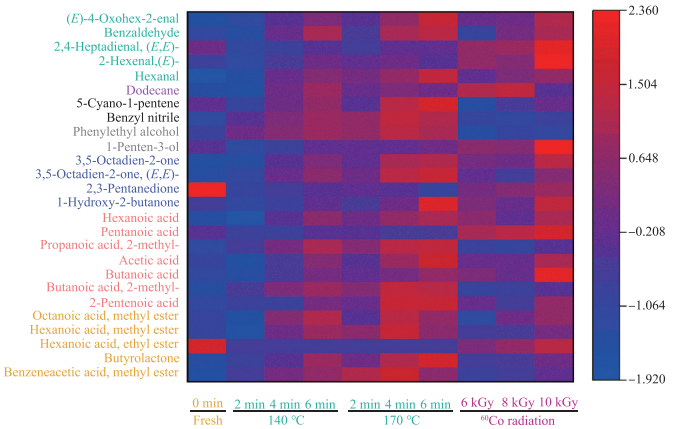


Fig. 6 Heatmap of the volatile compounds identified in rape bee pollen.

Statistical analysis showed significant differences ($P < 0.05$) in the content of volatile compounds originating from lipid oxidation between fresh samples and those treated with SHS and ^{60}Co radiation. Identified as the dominant volatile compound, acetic acid is known to be produced primarily by the breakdown of fatty acids. This phenomenon leads to the generation of a pungent odor at high concentrations. Feng et al.[43] confirmed the similar result that thermal processes such as high-temperature spray drying of milk can significantly increase the concentration of acetic acid compared to freeze-drying due to the complex thermal reactions induced by high temperature. Additionally, the formation of those organic acids may be derived from the further oxidation of the corresponding fatty aldehydes[44]. The increase of acetic acid and hexanoic acid concentration after treatment can give bee pollen a greasy taste and rancid flavor. Hexanoic acid, characterized by a penetrating, goaty, sweaty and cheesy aroma, also influences the sensory perception of products[45]. According to López et al.[46], aldehydes are the most abundant group of volatiles in products associated with rancid nuts. It was found that the aldehyde content of the ^{60}Co group irradiated pollen was higher than that of the SHS group. The susceptibility of irradiated samples to lipid oxidation is closely related to the nature, proportion, degrees of saturation in fatty acids[5]. Hexanal, produced as a result of the autoxidation of linoleic acid (a compound highly susceptible to oxidation) contributes to giving rape bee pollen a characteristic fatty and grassy aroma. Hexanal is widely recognized as a marker of lipid oxidation, which may be initiated by free radicals generated as a result of radiation or by radiation-catalyzed oxidation of PUFA and lipids containing the *cis* structure, *cis*-1,4-pentadiene. Both reactions produce lipid hydrogen peroxides, which decompose to form alcohols, aldehydes, and ketones, giving strong fatty flavors and cause food spoilage[5]. It should be emphasized that radiation and thermal oxidation processes proceeded through different mechanisms: with high temperatures favoring the formation of oleic acid-derived aldehydes, while radiation induces the formation of a mixture of oleic and linoleic acids derived aldehydes[44]. Furthermore, carbonyl groups formed by oxidative modification of proteins can lead to the formation of aldehydes, such as benzaldehyde through the Strecker degradation reaction, which may be another main cause of increased aldehydes contents[47]. Juan-Polo et al.[44] also found that the aldehydes signal increased due to both UV radiation or heating. An interesting finding is that hexanoic acid, ethyl ester was the only volatile compound detected that exhibited a decrease in concentration after ^{60}Co treatment and was not detectable in SHS treated samples. Although high temperatures have been reported to be detrimental to the stability of esters, there were significant trends for the content of other esters to increase after treatment[45]. However, there is no evidence on the effects of SHS treatment and radiation on ester chemical groups in food, which requires further research. Additionally, compounds such as 1-penten-3-ol, 2,3-pentanedione also give rape bee pollen a unique nutty, roasted and slightly irritating scent[48]. These findings confirmed that SHS treatment may be more effective in inhibiting lipid oxidation of rape bee pollen compared to ^{60}Co radiation treatment, highlighting the complexity of the impact of different treatment methods on the volatile compound profile and their consequences for rape bee pollen quality and stability.

4. Conclusion

Based on the conducted research, it was found that both SHS and ^{60}Co radiation treatment effectively reduced the microbial load to reach the safe levels for rape bee pollen. ^{60}Co radiation achieved complete inactivation of PPO at a dose of 6 kGy while SHS had a significant inactivation effect on POD and PPO. After SHS treatment at 140 °C for 2 min. the relative activity of POD and PPO showed significant reduction of 25.97% and 85.68%, respectively.

Table 3
Volatile compounds identified in rape bee pollen with different treatment methods.

Compounds	Name	RI	Fresh	140 °C			170 °C			^{60}Co radiation		
				2 min	4 min	6 min	2 min	4 min	6 min	6 kGy	8 kGy	10 kGy
Esters												
1	Benzeneacetic acid, methyl ester	1 753	91.24 ± 14.04^{f}	203.01 ± 5.68^{ef}	326.19 ± 47.90^{cd}	405.27 ± 25.07^{bc}	462.81 ± 101.74^{ab}	546.07 ± 38.05^{a}	392.82 ± 32.02^{bc}	199.84 ± 11.66^{ef}	203.76 ± 19.75^{ef}	268.90 ± 102.85^{de}
2	Butyrolactone	1 203	143.69 ± 21.06^{f}	200.92 ± 6.41^{de}	248.08 ± 27.61^{d}	372.52 ± 39.87^{bc}	291.17 ± 3.47^{cd}	410.25 ± 86.90^{ab}	471.19 ± 1.43^{a}	209.89 ± 9.58^{de}	226.60 ± 44.42^{de}	282.39 ± 71.02^{cd}
3	Hexanoic acid, ethyl ester	1 229	67.66 ± 7.24^{a}	0	0	0	0	0	0	24.52 ± 1.44^{c}	36.01 ± 5.00^{c}	52.49 ± 11.55^{b}
4	Hexanoic acid, methyl ester	1 179	1 946.46 ± 432.76^{cd}	907.94 ± 106.29^{e}	3 166.70 ± 440.36^{bc}	4 206.90 ± 95.67^{ab}	3 844.61 ± 941.32^{ab}	5 116.25 ± 341.95^{a}	3 864.16 ± 32.50^{ab}	2 188.34 ± 98.24^{cd}	2 368.82 ± 376.43^{c}	3 347.66 ± 941.12^{bc}
5	Octanoic acid, methyl ester	1 394	191.29 ± 21.79^{bcd}	79.76 ± 29.75^{d}	422.04 ± 45.29^{abcd}	544.29 ± 20.70^{ab}	273.84 ± 26.59^{abcd}	577.71 ± 31.68^{a}	481.09 ± 4.04^{abc}	136.52 ± 23.00^{cd}	353.05 ± 116.07^{abcd}	472.64 ± 36.80^{abc}
	Total esters		2 440.35 ± 496.89^{ef}	1 391.63 ± 148.13^{f}	4 163.01 ± 561.16^{bcde}	5 528.98 ± 181.31^{ab}	4 872.43 ± 1 073.12^{abcd}	6 650.28 ± 498.58^{a}	5 209.26 ± 69.99^{abc}	2 759.11 ± 143.92^{def}	3 188.24 ± 561.67^{cdef}	4 424.08 ± 1 163.34^{bcde}
Acids												
6	2-Pentenoic acid	1 780	30.17 ± 4.43^{e}	34.79 ± 8.42^{bc}	34.58 ± 6.06^{bc}	53.50 ± 28.31^{abc}	48.03 ± 12.19^{bc}	76.56 ± 9.42^{a}	78.34 ± 3.78^{a}	46.61 ± 2.56^{bc}	37.33 ± 4.51^{bc}	62.15 ± 14.96^{ab}
7	Butanoic acid, 2-methyl-	1 423	638.74 ± 76.21^{e}	1 077.07 ± 6.89^{d}	1 546.06 ± 159.76^{c}	1 928.14 ± 34.17^{ab}	1 672.38 ± 302.98^{bc}	2 170.84 ± 140.96^{a}	2 148.21 ± 39.98^{a}	887.96 ± 23.50^{de}	905.37 ± 146.64^{de}	1 175.91 ± 261.65^{d}
8	Butanoic acid	1 307	86.35 ± 9.68^{bc}	75.84 ± 2.83^{c}	104.77 ± 13.86^{bc}	131.82 ± 2.48^{bc}	118.11 ± 13.92^{bc}	131.10 ± 0.88^{bc}	149.34 ± 4.17^{ab}	136.97 ± 0.16^{abc}	122.91 ± 13.36^{bc}	202.33 ± 88.51^{a}
9	Acetic acid	1 438	16 195.39 ± 2 101.70^{de}	12 816.19 ± 450.18^{f}	18 464.89 ± 2 058.19^{cde}	23 944.99 ± 339.07^{bc}	19 113.19 ± 2 902.74^{cde}	26 737.21 ± 1 405.16^{ab}	31 344.34 ± 368.75^{a}	21 260.63 ± 246.20^{bcd}	21 429.07 ± 390.11^{bcd}	27 182.39 ± 6 261.72^{ab}
10	Propanoic acid, 2-methyl-	1 568	300.71 ± 38.31^{f}	446.65 ± 6.60^{ef}	641.48 ± 70.66^{cd}	811.48 ± 13.17^{ab}	689.35 ± 131.03^{bc}	887.58 ± 59.25^{a}	931.94 ± 17.46^{a}	395.99 ± 11.09^{ef}	390.11 ± 72.06^{ef}	503.58 ± 132.33^{de}
11	Pentanoic acid	1 565	36.11 ± 7.10^{b}	22.17 ± 0.82^{b}	25.55 ± 2.60^{b}	34.16 ± 2.37^{b}	34.27 ± 11.90^{b}	30.34 ± 3.80^{b}	33.99 ± 2.42^{b}	77.54 ± 9.17^{a}	87.25 ± 23.62^{a}	103.34 ± 25.68^{a}
12	Hexanoic acid	1 839	279.82 ± 26.16^{bc}	254.38 ± 5.65^{c}	341.60 ± 27.49^{abc}	410.82 ± 1.33^{a}	372.40 ± 53.65^{ab}	412.62 ± 23.68^{a}	436.98 ± 20.16^{a}	403.67 ± 84.33^{a}	350.00 ± 59.59^{abc}	437.34 ± 83.97^{a}
	Total acids		17 567.29 ± 3 253.91^{d}	14 727.09 ± 481.39^{d}	21 158.93 ± 2 338.62^{cd}	27 314.91 ± 420.90^{abc}	22 047.73 ± 3 248.41^{bcd}	30 446.26 ± 1 643.15^{ab}	35 123.14 ± 456.72^{a}	23 209.37 ± 377.01^{bcd}	23 322.04 ± 709.89^{bcd}	29 667.04 ± 6 784.85^{abc}
Ketones												
13	1-Hydroxy-2-butanone	1 373	54.17 ± 9.03^{cd}	42.48 ± 1.98^{d}	52.55 ± 6.92^{cd}	68.96 ± 0.78^{cd}	58.71 ± 6.19^{cd}	74.86 ± 6.32^{cd}	129.74 ± 3.08^{a}	83.49 ± 3.50^{bc}	69.55 ± 6.25^{cd}	116.61 ± 42.97^{ab}
14	2,3-Pentanedione	1 037	290.14 ± 26.14^{a}	92.27 ± 2.39^{e}	110.09 ± 12.00^{de}	126.09 ± 3.41^{de}	134.29 ± 18.21^{cde}	124.27 ± 8.60^{de}	87.70 ± 1.91^{e}	156.92 ± 1.15^{bcd}	178.57 ± 22.01^{bc}	198.31 ± 51.65^{b}
15	3,5-Octadien-2-one, (*E*,*E*)-	1 517	3 493.01 ± 515.13^{g}	4 067.56 ± 42.01^{fg}	6 079.19 ± 599.82^{cd}	7 482.31 ± 125.89^{bc}	6 435.82 ± 1 266.69^{cd}	8 958.17 ± 539.79^{ab}	9 670.23 ± 231.55^{a}	5 713.70 ± 20.59^{cde}	4 860.04 ± 792.37^{efg}	7 226.26 ± 1 808.27^{bc}
16	3,5-Octadien-2-one	1 562	1 374.04 ± 250.59^{e}	1 454.82 ± 5.09^{de}	2 142.64 ± 218.51^{cde}	2 645.47 ± 46.74^{abc}	2 234.38 ± 459.95^{cd}	3 126.05 ± 171.40^{ab}	3 186.81 ± 33.47^{ab}	2 500.54 ± 60.60^{abc}	2 435.75 ± 395.71^{bc}	3 291.36 ± 854.49^{a}
	Total ketones		5 211.36 ± 800.89^{d}	5 657.13 ± 51.47^{d}	8 384.47 ± 837.25^{bcd}	10 322.83 ± 176.82^{abc}	8 863.20 ± 1 751.04^{abcd}	12 284.11 ± 726.11^{ab}	13 074.48 ± 270.01^{a}	8 454.65 ± 85.84^{bcd}	7 543.91 ± 1 216.34^{cd}	10 832.54 ± 2 757.38^{abc}
Aldehydes												
17	Hexanal	1 057	388.34 ± 57.35^{c}	477.52 ± 77.44^{c}	662.88 ± 105.16^{b}	764.90 ± 73.04^{ab}	709.21 ± 81.53^{b}	806.20 ± 37.63^{ab}	926.54 ± 7.30^{a}	658.89 ± 45.37^{b}	746.18 ± 92.05^{b}	816.81 ± 50.86^{ab}
18	2-Hexenal, (*E*)-	1 211	843.10 ± 115.16^{cd}	679.36 ± 92.81^{d}	968.18 ± 104.18^{bcd}	1 168.48 ± 31.53^{bcd}	1 095.40 ± 346.01^{bcd}	1 175.67 ± 56.99^{bcd}	1 024.82 ± 3.55^{bcd}	1 459.23 ± 7.98^{b}	1 329.84 ± 107.98^{bc}	2 021.43 ± 602.91^{a}
19	2,4-Heptadienal, (*E*,*E*)-	1 487	1 712.90 ± 214.10^{cd}	834.55 ± 1.76^{f}	1 093.95 ± 89.13^{ef}	1 354.79 ± 4.77^{def}	1 144.11 ± 203.22^{b}	1 499.20 ± 63.98^{de}	1 544.34 ± 21.74^{de}	2 245.51 ± 2.35^{bc}	2 400.40 ± 254.14^{b}	3 287.65 ± 691.22^{a}
20	Benzaldehyde	1 513	116.40 ± 20.27^{de}	91.09 ± 6.09^{e}	185.62 ± 43.47^{bcd}	266.87 ± 11.22^{ab}	152.55 ± 50.40^{cde}	269.21 ± 29.75^{ab}	286.45 ± 13.11^{a}	117.47 ± 16.19^{cde}	201.80 ± 65.07^{abc}	269.75 ± 52.63^{ab}
21	(*E*)-4-Oxohex-2-enal	1 745	23.88 ± 3.84^{e}	32.26 ± 1.02^{de}	55.27 ± 6.45^{cde}	75.14 ± 6.61^{abc}	54.96 ± 10.80^{cde}	87.08 ± 6.68^{abc}	107.69 ± 31.72^{a}	60.98 ± 8.81^{cd}	67.65 ± 12.60^{bc}	97.94 ± 23.99^{ab}
	Total aldehydes		3 084.62 ± 410.72^{bc}	2 114.78 ± 179.12^{c}	2 965.91 ± 348.39^{bc}	3 630.18 ± 127.17^{bc}	3 156.23 ± 691.96^{bc}	3 837.37 ± 195.03^{bc}	3 889.83 ± 77.42^{bc}	4 542.09 ± 80.70^{ab}	4 745.86 ± 531.84^{ab}	6 493.57 ± 1 421.61^{a}
Alcohols												
22	1-Penten-3-ol	1 157	869.39 ± 136.38^{bcd}	610.77 ± 31.40^{d}	736.62 ± 114.59^{cd}	892.36 ± 14.41^{bcd}	884.19 ± 117.71^{bcd}	904.99 ± 58.07^{bcd}	962.58 ± 10.47^{bc}	1 135.87 ± 3.73^{b}	1 076.69 ± 146.72^{bc}	1 582.46 ± 373.77^{a}
23	Phenylethyl alcohol	1 900	68.37 ± 4.97^{e}	115.12 ± 7.45^{d}	148.17 ± 10.02^{c}	176.91 ± 0.74^{abc}	162.14 ± 20.90^{bc}	202.97 ± 16.74^{a}	179.06 ± 2.68^{ab}	30.25 ± 20.57^{f}	52.94 ± 8.45^{ef}	46.62 ± 12.10^{ef}
	Total alcohols		937.76 ± 141.35^{b}	725.89 ± 38.85^{b}	884.79 ± 124.61^{b}	1 069.27 ± 15.15^{ab}	1 046.33 ± 138.61^{ab}	1 107.96 ± 74.81^{ab}	1 141.63 ± 13.15^{ab}	1 166.12 ± 24.30^{ab}	1 129.62 ± 155.17^{ab}	1 629.08 ± 385.87^{a}
Nitrogenous compounds												
24	Benzyl nitrile	1 911	52.13 ± 8.75^{e}	107.32 ± 0.11^{d}	154.77 ± 11.75^{c}	189.07 ± 1.80^{bc}	174.40 ± 33.39^{bc}	224.95 ± 18.39^{a}	205.92 ± 6.83^{ab}	72.16 ± 12.82^{e}	62.67 ± 7.19^{e}	80.78 ± 17.64^{de}
25	5-Cyano-1-pentene	1 345	192.40 ± 25.34^{cde}	137.89 ± 0.14^{de}	221.97 ± 28.45^{bcd}	274.72 ± 9.33^{abc}	182.76 ± 100.52^{cde}	323.95 ± 34.23^{ab}	370.71 ± 8.97^{a}	99.90 ± 12.75^{e}	175.48 ± 16.43^{cde}	203.92 ± 118.03^{bcde}
Total nitrogenous compounds			244.54 ± 34.09^{bc}	245.21 ± 0.25^{bc}	376.74 ± 40.20^{abc}	463.79 ± 11.13^{ab}	357.16 ± 133.91^{abc}	548.90 ± 52.62^{a}	576.63 ± 15.80^{a}	172.06 ± 25.57^{c}	238.15 ± 23.62^{bc}	284.70 ± 135.67^{bc}
Alkane												
26	Dodecane	1 187	264.84 ± 9.37^{d}	256.19 ± 67.08^{a}	374.81 ± 80.19^{d}	437.41 ± 97.23^{b}	357.80 ± 103.10^{c}	387.00 ± 28.69	370.11 ± 63.97	451.45 ± 113.19	469.06 ± 368.15	341.23 ± 75.98

Note: Data are represented as the mean ± standard deviation (n = 3). Different lowercase letters in the same row mean significantly different ($P < 0.05$). The contents of VOCs identified were calculated by comparing their peak areas to that of 2-nonanone (0.205 mg/mL).

Additionally, the use of ^{60}Co radiation led to a significant improvement in BI and Δ*E* compared to samples treated with the SHS. SHS treatment induced visible changes in shape and surface of rape bee pollen, including the gradual enlargement and deepening of cracks in the germ opening, which can increase the effectiveness of the digestion and consequently bioavailability. The results of fatty acids confirmed that SHS treatment inhibited lipid oxidation processes more effectively compared to ^{60}Co radiation treatment, suggesting that SHS may be a more suitable method to protect lipids in pollen from degradation. The MDA levels were generally higher in treated samples compared to fresh rape bee pollen. Besides, the profile of volatile compounds also revealed that samples treated with ^{60}Co radiation were characterized by an increased content of aldehydes compared to SHS samples, which intensified the perception of fat flavor. These results indicate that SHS is an effective alternative processing technology compared to traditional ^{60}Co radiation treatment. SHS under 140 ℃ for 2 min was the most suitable to inactivate the microorganisms and enzymes in rape bee pollen with minimal lipid oxidation. It has great potential for bee pollen quality improvement and the findings will provide an effective theoretical basis for the development of the bee pollen industry.

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.

Acknowledgements

This work was supported by National Natural Science Foundations of China (32472396, 31871861 and 31501548), The Apicultural Industry Technology System (NCYTI-43-KXJ17), and The Science and Technology Innovation Project of Chinese Academy of Agricultural Sciences (CAAS-ASTIP-2015-IAR). The authors also would like to express their gratitude to the anonymous referees for their valuable comments and suggestions.

Reference

[1] Y.X. Bi, J.B. Ni, X.F. Xue, et al., Effect of different drying methods on the amino acids, *α*-dicarbonyls and volatile compounds of rape bee pollen, Food Sci. Hum. Wellness 13 (2024) 517-527. https://doi.org/10.26599/FSHW.2022.9250045.

[2] K.J. Li, J.Q. Zhao, W.J. Yu, et al., Deep enzymatic hydrolysis of bee pollen with multiple enzymes: improves nutrient release and potential biological activity of bee pollen, Food Biosci. 62 (2024) 105001. https://doi.org/10.1016/j.fbio.2024.105001.

[3] V. Aylanc, S.I. Falcão, S. Ertosun,et al., From the hive to the table: nutrition value, digestibility and bioavailability of the dietary phytochemicals present in the bee pollen and bee bread, Trends Food Sci. Technol. 109 (2021) 464-481. https://doi.org/10.1016/j.tifs.2021.01.042.

[4] Y.X. Bi, S. Zielinska, J.B. Ni, et al., Effects of hot-air drying temperature on drying characteristics and color deterioration of rape bee pollen, Food Chem.: X 16 (2022) 100464. https://doi.org/10.1016/j.fochx.2022.100464.

[5] S. Panseri, F. Arioli, R. Pavlovic, et al., Impact of irradiation on metabolomics profile of ground meat and its implications toward food safety, LWT-Food Sci. Technol. 161 (2022) 113305. https://doi.org/10.1016/j.lwt.2022.113305.

[6] J.J. Fang, C.X. Liu, C.L. Law, et al., Superheated steam processing: an emergingtechnology to improve food quality and safety, Crit. Rev. Food Sci. Nutr. 63(27) (2022) 8720-8736. https://doi.org/10.1080/10408398.2022.2059440.

[7] A.N. Kim, M.S. Rahman, K.Y. Lee, et al., Superheated steam pretreatment of rice flours: gelatinization behavior and functional properties during thermal treatment, Food Biosci. 41 (2021) 101013. https://doi.org/10.1016/j.fbio.2021.101013.

[8] L.Z. Deng, C.H. Xiong, P.P. Sutar, et al., An emerging pretreatment technology for reducing postharvest loss of vegetables-a case study of red pepper (*Capsicum annuum* L.) drying, Dry. Technol. 40(8) (2022) 1620-1628. https://doi.org/10.1080/07373937.2022.2039934.

[9] H.W. Xiao, J.W. Bai, D.W. Sun, et al., The application of superheated steam impingement blanching (SSIB) in agricultural products processing-a review, J. Food Eng. 132 (2014) 39-47. https://doi.org/10.1016/j.jfoodeng.2014.01.032.

[10] C. Ban, D.H. Lee, Y. Jo, et al., Use of superheated steam to inactivate *Salmonella enterica* serovars Typhimurium and Enteritidis contamination on black peppercorns, pecans, and almonds, J. Food Eng. 222 (2018) 284-291. https://doi.org/10.1016/j.jfoodeng.2017.11.036.

[11] H. Woo Park, V.M. Balasubramaniam, D.R. Heldman, et al., Computational fluid dynamics analysis of superheated steam's impact on temperature and humidity distribution within enclosed dry food processing spaces, J. Food Eng. 360 (2024) 111718. https://doi.org/10.1016/j.jfoodeng.2023.111718.

[12] L.Z. Deng, P.P. Sutar, A.S. Mujumdar, et al., Thermal decontamination technologies for microorganisms and mycotoxins in low-moisture foods, Annu. Rev. Food Sci. Technol. 12 (2021) 287-305. https://doi.org/10.1146/annurev-food-062220-112934.

[13] A. Alfy, B.V. Kiran, G.C. Jeevitha, et al., Recent developments in superheated steam processing of foods - a review, Crit. Rev. Food Sci. Nutr. 56(13) (2016) 2191-2208. https://doi.org/10.1080/10408398.2012.740641.

[14] Y.L. Luo, Y.Z. Bi, R. Du, et al., The impact of freezing methods on the quality, moisture distribution, microstructure, and flavor profile of hand-grabbed mutton during long-term frozen storage, Food Res. Int. 173 (2023) 113346. https://doi.org/10.1016/j.foodres.2023.113346.

[15] Y.M. Hu, L.J. Wang, Z.G. Li, et al., Superheated steam treatment on wheat bran: enzymes inactivation and nutritional attributes retention, LWT-Food Sci. Technol. 91 (2018) 446-452. https://doi.org/10.1016/j.lwt.2018.01.086.

[16] Z.H. Geng, X. Huang, J. Wang, et al., Pulsed vacuum drying of pepper (*Capsicum annuum* L.): effect of high-humidity hot air impingement blanching pretreatment on drying kinetics and quality attributes, Foods 11(3) (2022) 318. https://doi.org/10.3390/foods11030318.

[17] V. Verma, N. Yadav, Acrylamide content in starch based commercial foods by using high performance liquid chromatography and its association with browning index, Curr. Res. Food Sci. 5 (2022) 464-470. https://doi.org/10.1016/j.crfs.2022.01.010.

[18] J. Wang, Y.X. Chen, L.L. Zhao, et al., Lipidomics reveals the molecular mechanisms underlying the changes in lipid profiles and lipid oxidation in rape bee pollen dried by different methods, Food Res. Int. 162 (2022) 112104. https://doi.org/10.1016/j.foodres.2022.112104.

[19] Y.M. Hu, W. Nie, X.Z. Hu, et al., Microbial decontamination of wheat grain with superheated steam, Food Control 62 (2016) 264-269. https://doi.org/10.1016/j.foodcont.2015.11.001.

[20] V.P. Valdramidis, A.H. Geeraerd, J.E. Gaze, et al., Quantitative description of *Listeria monocytogenes* inactivation kinetics with temperature and water activity as the influencing factors; model prediction and methodological validation on dynamic data, J. Food Eng. 76(1) (2006) 79-88. https://doi.org/10.1016/j.jfoodeng.2005.05.025.

[21] H.R. Wang, L.L. Wang, L.T. Tong, et al., Effect of superheated steam inactivation on naturally existent microorganisms and enzymes of highland barley, Int. J. Food Sci. Technol. 54(8) (2019) 2570-2577. https://doi.org/10.1111/ijfs.14168.

[22] L.Z. Deng, Y. Tao, A.S. Mujumdar, et al., Recent advances in non-thermal decontamination technologies for microorganisms and mycotoxins in low-moisture foods, Trends Food Sci. Technol. 106 (2020) 104-112. https://doi.org/10.1016/j.tifs.2020.10.012.

[23] R. Ravindran, A.K. Jaiswal, Wholesomeness and safety aspects of irradiated foods, Food Chem. 285 (2019) 363-368. https://doi.org/10.1016/j.foodchem.2019.02.002.

[24] National Standards of People's Republic of China. 2016. Pollen, GB 31636–2016. Beijing.

[25] A.L.T.d. Jesus, T.S. Leite, M. Cristianini, High isostatic pressure and thermal processing of açaí fruit (*Euterpe oleracea* Martius): effect on pulp

color and inactivation of peroxidase and polyphenol oxidase, Food Res. Int. 105 (2018) 853-862. https://doi.org/ 10.1016/j.foodres.2017.12.013.

[26] L.Z. Deng, Z. Pan, A.S. Mujumdar, et al., High-humidity hot air impingement blanching (HHAIB) enhances drying quality of apricots by inactivating the enzymes, reducing drying time and altering cellular structure, Food Control 96 (2019) 104-111. https://doi.org/10.1016/j.foodcont.2018.09.008.

[27] K. Marszałek, M. Mitek, S. Skąpska, The effect of thermal pasteurization and high pressure processing at cold and mild temperatures on the chemical composition, microbial and enzyme activity in strawberry purée, Innov. Food Sci. Emerg. Technol. 27 (2015) 48-56. https://doi.org/10.1016/j.ifset.2014.10.009.

[28] M. Constantinovici, D. Oancea, T. Zaharescu, Gamma irradiation effect on the enzymatic activities of horseradish and apple peroxidases, Radiat. Phys. Chem. 78(1) (2009) 33-36. https://doi.org/10.1016/j.radphyschem.2008.07.008.

[29] A. Rodacka, J. Gerszon, M. Puchala, et al., Radiation-induced inactivation of enzymes-Molecular mechanism based on inactivation of dehydrogenases, Radiat. Phys. Chem. 128 (2016) 112-117. https://doi.org/10.1016/j.radphyschem.2016.05.022.

[30] H. Wang, X. M. Fang, P. P. Sutar, et al., Effects of vacuum-steam pulsed blanching on drying kinetics, colour, phytochemical contents, antioxidant capacity of carrot and the mechanism of carrot quality changes revealed by texture, microstructure and ultrastructure, Food Chem. 338 (2021) 127799. https://doi.org/10.1016/j.foodchem.2020.127799.

[31] R. Sehrawat, P.K. Nema, B.P. Kaur, Effect of superheated steam drying on properties of foodstuffs and kinetic modeling, Innov. Food Sci. Emerg. Technol. 34 (2016) 285-301. https://doi.org/10.1016/j.ifset.2016.02.003.

[32] J. Wang, X.M. Fang, A.S. Mujumdar, et al., Effect of high-humidity hot air impingement blanching (HHAIB) on drying and quality of red pepper (*Capsicum annuum* L.), Food Chem. 220 (2017) 145-152. https://doi.org/10.1016/j.foodchem.2016.09.200.

[33] H. Wang, Q. Zhang, A.S. Mujumdar, et al., High-humidity hot air impingement blanching (HHAIB) efficiently inactivates enzymes, enhances extraction of phytochemicals and mitigates brown actions of chili pepper, Food Control 111 (2020) 107050. https://doi.org/10.1016/j.foodcont.2019.107050.

[34] T.J. Jiang, S.S. Luo, Q.P. Chen, et al., Effect of integrated application of gamma irradiation and modified atmosphere packaging on physicochemical and microbiological properties of shiitake mushroom (*Lentinus edodes*), Food Chem. 122(3) (2010) 761-767. https://doi.org/10.1016/j.foodchem.2010.03.050.

[35] Y. Liu, J.P. Chen, X.F. Wu, et al., Insights into the effects of γ-irradiation on the microstructure, thermal stability and irradiation-derived degradation components of microcrystalline cellulose (MCC), RSC Adv. 5(43) (2015) 34353-34363. https://doi.org/10.1039/C5RA03300D.

[36] Y. Wang, Y. T. Jiang, J. X. Cao, et al., Study on lipolysis-oxidation and volatile flavour compounds of dry-cured goose wit nt curing salt content during production, Food Chem. 190 (2016) 33-40. https://doi.org/10.1016/j.foodchem.2015.05.048.

[37] P. Yodkaew, N. Chindapan, S. Devahastin, Influences of superheated steam roasting and water activity control as oxidation mitigation methods on physicochemical properties, lipid oxidation, and free fatty acids compositions of roasted rice, J. Food Sci. 82(1) (2017) 69-79. https://doi.org/10.1111/1750-3841.13557.

[38] E. Bravi, O. Marconi, V. Sileoni, et al., Determination of free fatty acids in beer, Food Chem. 215 (2017) 341-346. https://doi.org/10.1016/j.foodchem.2016.07.153.

[39] S.Y. Ha, H.M. Joo, S.H. Kang, et al., Effect of ultraviolet irradiation on the production and composition of fatty acids in plankton in a sub-Antarctic environment, J. Oceanogr. 70(1) (2014) 1-10. https://doi.org/10.1007/s10872-013-0207-3.

[40] Q. Zhang, X. C. Chen, Y. T. Ding, et al., Diversity and succession of the microbial community and its correlation with lipid oxidation in dry-cured black carp (*Mylopharyngodon piceus*) during storage, Food Microbiol. 98 (2021) 103686. https://doi.org/10.1016/j.fm.2020.103686.

[41] T.C. Huang, C.T. Ho, H.Y. Fu, Inhibition of lipid oxidation in pork bundles processing by superheated stream frying, J. Agric. Food Chem. 52(10) (2004) 2924-2928. https://doi.org/10.1021/jf030641o.

[42] K.L. Liu, Y. Liu, F.S. Chen, Effect of gamma irradiation on the physicochemical properties and nutrient contents of peanut, LWT-Food Sci. Technol. 96 (2018) 535-542. https://doi.org/10.1016/j.lwt.2018.06.009.

[43] D. Feng, J. Wang, X.J. Ji, et al., HS-GC-IMS detection of volatile organic compounds in yak milk powder processed by different drying methods, LWT-Food Sci. Technol. 141 (2021) 110855. https://doi.org/10.1016/j.lwt.2021.110855.

[44] A. Juan-Polo, A. Beltrán Sanahuja, M. Monedero Prieto, et al., Impact of UV-light irradiation on sensory properties, volatile, fatty acid, and tocopherol composition of peanuts (*Arachis hypogaea* L.), LWT-Food Sci. Technol. 173 (2023) 114247. https://doi.org/10.1016/j.lwt.2022.114247.

[45] J.B. Ni, Y.X. Bi, S.K. Vidyarthi, et al., Non-thermal electrohydrodynamic (EHD) drying improved the volatile organic compounds of lotus bee pollen *via* HS-GC-IMS and HS-SPME-GC-MS, LWT-Food Sci. Technol. 176 (2023) 114480. https://doi.org/10.1016/j.lwt.2023.114480.

[46] P.L. López, M.A. Marchesino, N.R. Grosso, et al., Comparative study of accelerated assays for determination of equivalent days in the shelf life of roasted high oleic peanuts: chemical and volatile oxidation indicators in accelerated and room temperature conditions, Food Chem. 373 (2022) 131479. https://doi.org/10.1016/j.foodchem.2021.131479.

[47] F.J. Hidalgo, R. Zamora, Formation of phenylacetic acid and benzaldehyde by degradation of phenylalanine in the presence of lipid hydroperoxides: new routes in the amino acid degradation pathways initiated by lipid oxidation products, Food Chem.: X 2 (2019) 100037. https://doi.org/10.1016/j.fochx.2019.100037.

[48] K. Lu, L. Liu, J.W. Zi, et al., New insights from flavoromics on different heating methods of traditional fermented shrimp paste: the volatile components and metabolic pathways, LWT-Food Sci. Technol. 168 (2022) 113880. https://doi.org/10.1016/j.lwt.2022.113880.