Lipids Oxidized Volatile Compounds Profuced in Pine Pollen as affected by Electron-beam Sterilization and Ultra-high Temperature Sterilization

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\textbf{ABSTRACT}

Pine pollen is rich in unsaturated fatty acids. However, unsaturated fatty acids are oxidized during processing. The oxidation of lipids may affect the odor of pine pollen. Different sterilization methods have different effects on lipid oxidation. In this study, we found that electron-beam sterilization is superior to ultra-high temperature sterilization in odor preservation. Using gas chromatography–mass spectrometry, volatile components were identified in electron-beam sterilization and ultra-high temperature sterilization processed pine pollen. Furthermore, the loss of vitamin C and polyphenols in the processing aggravated lipid oxidation, which made the odor of pine pollen worse. Moreover, lipid oxidase can accelerate lipid oxidation, thus affecting the odor of pine pollen, which was not conducive to the preservation of pine pollen. The results suggested that the components of unpleasant odor were identified as volatile aldehydes and volatile acids, and the odor was mainly produced by the oxidation of linoleic acid.

\textbf{KEYWORDS}

Electron-Beam sterilization; Ultra-high temperature sterilization; GC-MS; Lipid oxidation; Aroma retention

\section*{Introduction}

Pine pollen is the male spore of pine trees. It is a kind of functional food and also a traditional Chinese herbal medicine.\textsuperscript{[1]} It is rich in many kinds of amino acids, minerals, vitamins, enzymes, flavonoids and other reducing ingredients required by human body.\textsuperscript{[2]} Pine pollen has been used as medicine and food for thousands of years.\textsuperscript{[3]} At present, in the process of processing and storage, the pine pollen products treated by ultra-high temperature sterilization (UHT) have different degrees of lipid oxidation odor. The pine pollen products on the market were mainly sporoderm-broken pine pollen. The main morphological change in sporoderm-broken pollen is that air bags are separated from pollen particles, and most of the main body of pollen particles is broken. Notably, during pine pollen processing, it is often required to break the sporoderm in order to increase the availability and accessibility of the effective ingredients in the spores, which correspondingly exposes these ingredients to light, heat, moisture and oxygen.\textsuperscript{[4]} This treatment may lead to lipid oxidation and rancidity of the pollen during storage. The lipids are oxidized to hydrogen peroxide. The rate of oxidation is relatively slow. However, oxidation is accelerated by the promotion of light and heat,
followed by decomposition into aldehydes, ketones and lower fatty acids. The decomposition and polymerization of hydroperoxide, followed by the formation of free radicals and complex oxidation products, causes the deterioration of odor.

Ultra-high temperature sterilization (UHT) is a sterilization method that sterilizes microorganisms and preserves the active ingredients in products. However, UHT does not effectively kill enzymes\(^{[5,6]}\). During the UHT process, various factors such as water and oxygen in the material will destroy the natural antioxidant active ingredients in the food such as VC, VE, polyphenols, etc., resulting in a decrease in the antioxidant stability of the UHT sterilized product, thus accelerating the lipids oxidation process, resulting in the rancidity of pollen products and the lipid oxidation odor.\(^{[7,8]}\). Electron-beam irradiation-(EBI) sterilization is another kind of irradiation sterilization technology, which can effectively prolong the shelf life of food. High-energy electron beam directly acts on and destroys DNA in living biological cells, and when it acts on food, it rapidly irradiates and excites its outer orbital electrons to produce active radicals such as -H and -OH through the irradiation of water and small molecules, which interact with intranuclear substances and cross-link to kill harmful organisms, thus extending the shelf life of food.\(^{[9–11]}\)

The purpose of this study was to investigate the causes of lipid oxidation odor problems in products produced from raw materials treated with ultra-high temperature instantaneous sterilization process, determined the indicators and range of indicators related to the generation of lipid oxidation odor, and judged whether electron beam sterilization was more suitable for the sterilization of pine pollen to improve product quality and shelf life. In addition, it was necessary to determine the substances that produce lipid oxidation odor and the causes of production, and to find the influencing factors that lead to lipid oxidation odor in products.

**Materials and methods**

**Pine pollen sample details**

Pine pollen samples were donated by New Era Health Industry Group Co. Ltd (Shandong Province, China). Pine pollen samples (UHT-PP: ultra-high temperature sterilization sporoderm-broken pine pollen; EBI-PP::electron-beam irradiation sterilization sporoderm-broken pine pollen) were generally processed according to the scheme shown in Figure 1 and Figure 2. The moisture content of the collected pine pollen was reduced from 30% to 10% after sun-drying, then air-dried and further reduced to 7% before sterilization. During processing, the pine pollen was sterilized by ultra-high temperature or electron beam radiation to inactivate endogenous and exogenous enzymes to improve microbial safety, and then the sterilized pine pollen was crushed. In particular, a 15% suspension of pine pollen was subjected to ultra-high temperature heat treatment using an ultra-high temperature heat treatment machine. First, the sample solution was heated to 80°C and then flowed into the pipeline at 120°C. The pipeline pressure was adjusted to ensure that the sample remained in the liquid state at 120°C. The sterilization time was controlled by varying the residence time at 120°C in the pipeline. Finally, the processed pine pollen is made into pine pollen products (powder or tablets). The electron beam sterilization process is carried out in the workshop of Canada Blue Rich Medical

![Figure 1](image-url)  
**Figure 1.** Scheme of the sample preparation of pine pollen products (UHT-PP)*. *WC: water content
Technology (Shandong) Co. The electron beam energy of the device was 10emv, the beam intensity was 2 mA, the electron beam scanning angle was ≥ 30°, and the electron beam radiation scanning dose was 10kGy. Before scanning, the pine pollen was packed in transparent plastic bags sealed with each bag containing 100 g, and each measurement setup was repeated 3 times. The actual length, width and height of the sample should be within the scanning penetration range and put into the scanning line for cyclic irradiation sterilization.

**Degree of oxidation in pine pollen with different treatments (TBA method)**

Took 2.0g of pine pollen sample into beaker, added 50 mL 7.5% trichloroacetic acid (m/m 7.5%) solution containing 0.1% EDTA (m/m) into beaker, mixed well, filtered turbid solution to be clear and transparent, aspirated 5 ml of supernatant, mixed well with thiobarbituric acid solution of the same volume, heated in 92 °C constant temperature water bath for 45 min, and carried out reagent blank treatment at the same time. After heating, took it out and cooled it to room temperature, transfered it into a 20 ml centrifuge tube, centrifuged it at 6500 r/min for 5 min, then took the supernatant out to the tube, added 5 ml of chloroform and mixed it evenly, waited for the liquid to settle and separate, took the supernatant, compared it with the reagent blank, and measured the absorbance value of the sample at the wavelength of 532 nm and 600 nm respectively. The thiobarbituric acid (expressed in milligrams of malondialdehyde per 100 g pine pollen) value was calculated by the formula (2–1) as follows:

\[
TBA = \frac{(A_{532nm} - A_{600nm}) \times 72.06 \times 100}{155 \times 2}
\]  

(1)

\(A_{532nm}\) was the absorbance value measured at 532 nm, and \(A_{600nm}\) was the absorbance value measured at 600 nm. 72.06 was the molecular weight of malondialdehyde (MDA).
Electronic nose

The GC-Flash electronic nose system was used to analyze the principal components (PCA) of the pine pollen samples under different treatments, and the probe was manually selected based on the results to compare the differences in the radar plots of each sample.

Solid Phase Microextraction (SPME)

The extraction of the volatile components of the headspace of pine pollen (0.3 g with 5 mL water in 20 mL screw-cap vial) was performed in duplicate and accomplished automatically using a CombiPAL autosampler (Agilent Technologies, Santa Clara, CA, USA). The fiber used was coated with Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm film thickness, 1 cm long), acquired from Supelco (Sigma-Aldrich), which was inserted into the headspace of the sample and maintained for 60 min (60°C). Variables such as the type of fiber (polarity and thickness of the coating) and the extraction conditions (sample and headspace volumes, extraction time and temperature) had been previously tested in our laboratory in order to ensure reproducible and reliable results. The effectiveness of the SPME fiber used was periodically verified by testing its extraction efficiency with a reference sample of known composition. The fiber containing the extracted components was desorbed for 5 min in the injection port of a gas chromatograph model GC 7890A equipped with a mass selective detector 5975 C inert MSD with Triple Axis Detector (Agilent Technologies) and a computer operating with the ChemStation program.

Volatility analysis

Gas chromatographic conditions: carrier gas flow rate (high-purity helium): 1.0 mL/min, no splitting; column was a polar column DB-WAX with an initial temperature of 40 °C, maintained for 4 min, ramped up to 70 °C with a 6 °C/min program, maintained for 2 min, then ramped up to 230 °C with a 10 °C/min program, maintained for 8 min. Mass spectrometry conditions: full scan (scan mode) acquisition signal, ionization mode EI, electron attack energy of 70 eV; forward sample port temperature of 250 °C, ion source temperature of 230 °C, quadrupole temperature of 150 °C, scan range of 33–450 amu.

The quantitative method was internal standard method. After adding ultrapure water, added 50 μl internal standard (diluted to 1.0 × 10⁻⁴ or 1.0 × 10⁻⁵ concentration of 2-decanone by methanol) and mixed with vortex oscillator.

\[
m_i = \frac{m_0 \times A_i}{A_0 \times m_0}
\]

Among them, \(m_i\) was the content of volatile matter, \(μ g/g\); \(m_0\) was the content of internal standard matter, \(μ g\); \(m_0\) was the mass of pine pollen used in the experiment, g; \(A_i\) was the peak area of volatile matter; \(A_0\) was the peak area of internal standard matter.

Basic components of pine pollen with different treatments

For R-PP, EBI-PP and UHT-PP samples of total sugar (total sugar content detection kits), reducing sugar(reducing sugar content detection kits), fat (GB 5009.6 2016 in the second method), fatty acid (GB 5009.168 2016), protein (GB 5009.5 2016 in the first method), ash content (GB 5009.4 2016 in the first method), moisture content (GB 5009.3 2016 in the first method) and water activity (GB 5009.238 2016 in the second method) and other basic components of the determination, analyze and compare the content of each component.
Functional components of pine pollen with different treatments

Ascorbic acid VC (Nanjing built ascorbic acid test kit), total phenol content (T/AHFIA 055–2018), total flavone content (DB13/T 3851998) and other functional components of R-PP, electron-beam sterilized and heat sterilized pine pollen samples were determined.

Lipoxygenase (LOX) activity of pine pollen with different treatments

The detection method was in accordance with the steps in the Solarbio test kit for LOX activity in plant.

Color difference of pine pollen in different treatments based on digieye

DigiEye (VeriVide, UK) was used to analyze the color difference of pine pollen samples. Measure the color value with colorimeter, and record the CIE color value as L* (brightness), a* (red) and b* (yellow). The total color difference (ΔE) between the control and the treated sample was calculated using equation \( ΔE = (∆L^2 + ∆a^2 + ∆b^2)^{1/2} \).

Statistical analysis

SPSS 18.0 and design expert 8.0.6 software (including ANOVA variance analysis) were used for statistical analysis of data, and Excel software was used for mapping. Differences were considered significant at \( P < .05 \).

Results and discussion

Electronic nose

The odor and lipid oxidation were deeper in UHT-PP compared to EBI-PP. As seen in Figure 3, the principal component analysis of EBI-PP and R-PP did not differ significantly, but the differences between UHT-PP and R-PP were significant. The electronic nose analysis showed that the response values of the metal probe detectors in the UHT-PP group were significantly different from those of the R-PP and EBI-PP groups, where they were higher than those of the other two groups (i.e., 4.55-1-A, 50.54-2-A, 39.74-2-A, 38.24-1-A, 35.24-1-A), compared to the 4.98-2-A detectors for the UHT-PP group the opposite response. The results indicated that at least six volatile components underwent volume changes during UHT sterilization. The 10 kGy high-dose EBI sterilization had little effect on the odor of pine pollen, which was more suitable for pine pollen processing.

Different treatments on pine pollen color based on electronic eye analysis

As shown in Table 1 and Figure 4 below, there were no significant differences in the lightness (L*), blue-yellow value (b*) and total color change (ΔE) of pine pollen. The a* values of heat-sterilized pine pollen were significantly different compared to R-PP and EBI-PP. The L* and B* parameters of UHT pine pollen decreased with increasing a* parameters, but the changes were not significant. The results indicated that EBI sterilization could better protect the pigment fraction in pine pollen, while heat sterilization could lead to the decomposition or loss of pigments due to the contact between heat and water during the sterilization process.
Figure 3. The E-nose Radar distinguish between raw pine pollen (R-PP), ultra-high temperature instantaneous sterilization sporoderm-broken pine pollen (UHT-PP) and electron-beam irradiation sterilization sporoderm-broken pine pollen (EBI-PP). The odor composition of EBI-PP is completely different from the other two kinds of pine pollen.

Figure 4. The appearance between raw pine pollen (R-PP), ultra-high temperature instantaneous sterilization sporoderm-broken pine pollen (UHT-PP) and electron-beam irradiation sterilization sporoderm-broken pine pollen (EBI-PP).

Table 1. Color indices from different treatments of pine pollen.

| Type       | L* ±SD | a* ±SD  | b* ±SD  | ΔE ±SD |
|------------|--------|---------|---------|--------|
| R-PP       | 94.43 ± 0.12<sup>a</sup> | -2.15 ± 0.05<sup>a</sup> | 21.27 ± 0.17<sup>a</sup> | 0      |
| EBI-PP     | 93.70 ± 0.04<sup>b</sup> | -1.13 ± 0.07<sup>b</sup> | 22.44 ± 0.11<sup>b</sup> | 1.72 ± 0.03 |
| UHT-PP     | 89.31 ± 0.24<sup>c</sup> | 3.68 ± 0.03<sup>c</sup> | 35.89 ± 0.08<sup>c</sup> | 16.55 ± 0.06 |

Data were expressed by means±SD (n=3). Different letters in the same column indicate significantly different results (P ≤ 0.05). (UHT-PP: ultra-high temperature instantaneous sterilization sporoderm-broken pine pollen; EBI-PP: electron-beam irradiation sterilization sporoderm-broken pine pollen; R-PP: raw pine pollen)
Basic physical and (bio)chemical characterization of pine pollen samples

As can be seen from Table 2, total sugar content, fat content, protein content, ash content, moisture content and water activity were slightly decreased compared to R-PP, except for a slight increase in reducing sugar content. Fat content was significantly decreased in the UHT-PP group, which implies that after heat and water contact, the fat in pine pollen was oxidized compared to the R-PP and EBI-PP groups. The water activity of the UHT-sterilized pine pollen was lower compared to that of the Electron beam-sterilized pine pollen. When the water activity was too low, it could significantly promote the lipid oxidation of pine pollen.

According to Figure 5, its functional components such as vitamins, polyphenols, and flavonoids were lost to different degrees after EBI sterilization and heat sterilization, but heat sterilization caused a greater loss of functional components than EBI sterilization. During the production process, the reduction of reducing components would make the unsaturated fatty acids in pine pollen more susceptible to oxidation. Excessive loss of reducing substances during production would make the nutritional value of the product lower.

Table 2. (Bio)chemical and physical characterization of pine pollen samples.

| Type                        | R-PP      | EBI-PP    | UHT-PP    |
|-----------------------------|-----------|-----------|-----------|
| Total sugar content (mg/g)  | 100.61 ± 0.57<sup>a</sup> | 100.12 ± 0.77<sup>a</sup> | 99.01 ± 0.21<sup>b</sup> |
| Reducing sugar content (mg/g)| 4.24 ± 0.04<sup>a</sup> | 4.57 ± 0.10<sup>b</sup> | 16.38 ± 0.21<sup>c</sup> |
| Fat content (g/100 g)       | 8.42 ± 0.06<sup>a</sup> | 8.39 ± 0.09<sup>a</sup> | 8.07 ± 0.06<sup>b</sup> |
| Protein content (g/100 g)   | 12.26 ± 0.16<sup>a</sup> | 12.18 ± 0.23<sup>a</sup> | 12.10 ± 0.17<sup>a</sup> |
| Ash content (g/100 g)       | 2.80 ± 0.03<sup>a</sup> | 2.83 ± 0.03<sup>a</sup> | 2.88 ± 0.05<sup>a</sup> |
| Moisture content (%)        | 6.80 ± 0.14<sup>a</sup> | 6.21 ± 0.52<sup>a</sup> | 6.48 ± 0.08<sup>a</sup> |
| Water activity (Aw)         | 0.49 ± 0.02<sup>a</sup> | 0.47 ± 0.03<sup>a</sup> | 0.40 ± 0.03<sup>a</sup> |

Data were expressed by means±SD (n = 3). Different letters in the same row indicate significantly different results (P < 0.05). (UHT-PP: ultra-high temperature instantaneous sterilization sporoderm-broken pine pollen; EBI-PP: electron-beam irradiation sterilization sporoderm-broken pine pollen; R-PP: raw pine pollen).

Figure 5. Effects of different treatments on thiobarbituric acid value and Lipoxgenase activity of pine pollen. thiobarbituric acid value (A), Lipoxygenase activity (B), flavone content (C). Data were expressed by means ± standard deviations (n = 3). Values with different letters are significantly different (P < .05).(UHT-PP: ultra-high temperature instantaneous sterilization sporoderm-broken pine pollen; EBI-PP: electron-beam irradiation sterilization sporoderm-broken pine pollen; R-PP: raw pine pollen).
processing is also a cause of lipid oxidation odor of pine pollen. In general, EBI sterilization has less effect on the main components of pine pollen and basically allows the maximum retention of the nutritional components of pine pollen.

High temperature is an important factor affecting the odor of lipid oxidation of pine pollen. Under high temperature conditions, natural antioxidant active ingredients such as VC, VE and polyphenols are easily destroyed, and unsaturated fatty acids in food are more prone to oxidative decomposition, Maillard reaction, browning and product color deepening. Changing the food sterilization process from hot sterilization to cold sterilization, and replacing ultra-high temperature sterilization with irradiation sterilization is a suitable solution.

**Sterilization methods on thiobarbituric acid value and lipoxygenase activity of pine pollen**

As seen in Figure 6(a), the thiobarbituric acid values of UHT-PP were significantly higher than those of R-PP and EBI-PP. Before treatment, pine pollen contained 3.03 mg/100 g of lipid oxidation products. After treatment, the thiobarbituric acid value increased only by 0.011 mg/100 g. The difference was not significant. During the processing of pine pollen, the lipids undergo degradation, i.e., decomposition into carbonyl compounds, the thiobarbituric acid value of UHT-PP was 2.5 times higher than that of R-PP, which further suggests that UHT-PP is more susceptible to lipid oxidation. This may be due to the fact that heating not only leads to lipolysis (especially triacylglycerols) and accelerates the formation of lipid radicals, but also destroys natural antioxidants. The results indicate that high-dose EBI sterilization does not significantly promote lipid oxidation of pine pollen, but heat sterilization leads to severe oxidative deterioration and a drastic decrease in product quality.

As shown in Figure 6(b), the lipoxygenase activity in pine pollen reached 6.23 U/g, which was reduced to 3.92 U/g under the effect of heat sterilization, while the lipoxygenase activity in pine pollen after EBI sterilization was 1.4 U/g. The results indicate that EBI sterilization has a strong inhibitory effect on the lipoxygenase activity in pine pollen, which can effectively prevent the oxidative deterioration of pine pollen during the processing and The results showed that the sterilization of pine pollen by EBI inhibited the activity of lipoxygenase in pine pollen and effectively prevented the oxidative deterioration and odor of pine pollen during processing. During processing, both sterilization methods reduced the activity of lipoxygenase in pine pollen, indicating a lower degree of enzyme oxidation. Regardless of which sterilization method could not completely inactivate lipoxygenase, the oxidase inherent in crushed pollen could effectively contact with reaction substrates such as fatty acids to oxidize unsaturated fatty acids, so the pine pollen products were susceptible to lipoxygenase during the storage process.

![Figure 6](image_url)

*Figure 6.* Effects of different treatments on thiobarbituric acid value and Lipoxygenase activity of pine pollen. thiobarbituric acid value (A), Lipoxgenase activity (B) Data were expressed by means ± standard deviations (n = 3). Values with different letters are significantly different (p < .05). (UHT-PP: ultra-high temperature instantaneous sterilization sporoderm-broken pine pollen; EBI-PP: electron-beam irradiation sterilization sporoderm-broken pine pollen; R-PP: raw pine pollen).
LOX activity decreased and TBA values increased in the UHT sterilized group. This indicated that the lipid composition was more influenced by external factors than by internal LOX. The lipid oxidation odor of pine pollen products was mainly caused by enzymatic and non-enzymatic oxidation. The key to the control of enzymatic oxidation was to effectively inactivate the activity of oxidase. The key to non-enzymatic oxidation control was to control oxygen, moisture, light and metal ions associated with lipid oxidation. The product had a lipid oxidation odor, which was due to ineffective passivation of lipoxygenase, resulting in an enzymatic oxidation reaction. The results illuminated that the processing is not rational and promotes the non-enzymatic oxidation of lipids.

**Volatility analysis**

As can be seen from Table 3, the volatile components of untreated pine pollen, 10 kgy dose of electron beam sterilized pine pollen and heat sterilized pine pollen were 43, 51 and 45, respectively. Alkenes were the most abundant volatile components in pine pollen, with 76.06% and 77.36% in the EBI-PP and UHT-PP groups, respectively, and 71.26% in the UHT-PP group, respectively, but the total volatile components in the UHT-PP group were only about 50% of those in R-PP and EBI-PP. UHT caused the most important odorant substances in pine pollen to be severe loss, while Electron beam sterilization did not have such a strong effect. The total amount of volatile substances accounted for half of the R-PP. In UHT-treated pine pollen, more alkenes, ketones, esters, phenols and other substances were lost, while more aldehydes and volatile acids were added. The results showed that the greatest changes in aldehydes and hexanoic acid were observed after ultra-high temperature sterilization, with an increase in aldehydes and hexanoic acid in the ultra-high temperature sterilized PP group. Hexanal, 2-buty1-2-octenal (baked animal liver odor, associated with the Maillard reaction) and decanal (only in the UHT group) were commonly present in decayed meat, which is the same odor we previously described in degraded pine pollen samples.

Aldehydes and acids are usually associated with lipid oxidation. Alkenes and unsaturated fatty acids were oxidized and cracked in the process of processing, forming small molecules of volatile aldehydes and volatile acids. Organic acids such as acetic acid, pentanoic acid and hexanoic acid had been previously identified as possible tertiary products of lipid oxidation for almonds.\[14\]

Quantitation of headspace volatiles (e.g., hexanal) associated with rancidity had been used in some foods to better approximate development of rancid odor and aroma, such as almond,\[15\] oat flours\[16\] and potato crisps.\[17\] Under accelerated storage conditions, cleavage of linoleic hydroperoxides could result in production of hexanal, pentanal, 2-octenal, 3-octen-2-one, 2-hexenal, 2,4-decadienal, 2,4-nonenal and 2-heptenal.\[18\] All these aldehydes and ketones were identified in pine pollen headspace, especially hexanal, which was the most frequently used to indicate the level of lipid oxidation in foods.\[19\] Previous investigations had reported hexanal as the major aldehyde indicating lipid oxidation in lipid-containing or lipid-based food systems where oleic acid was present in significantly higher proportions compared to linoleic acid, as was the case in the present study. For example, hexanal was used to monitor lipid oxidation volatiles in meat and low-fat carrot and tomato products,\[20\] potato crisps,\[17\] wheat crackers\[21\] and olive oil.\[22\] Acetic acid, 2-buty1-2-octenaldehyde and decanal increased after processing, indicating that heat sterilization had certain influence on the aroma of pine pollen.\[23\]

The esters and alcohols in pine pollen also accounted for a part of its volatile compounds, which were 6.73% and 5.91% before treatment, respectively. The total content of esters and alcohols increased by 6.44% and 6.76%, respectively, after EBI sterilization, where alcohols increased after Electron beam irradiation, while heat sterilization reduced both substances to 4.42% and 5.83%, respectively. Alcohols have a distinct aromatic odor and are lost during processing.

For ketones, 2-octanone was the odor substance in baked food or fried soybean chips, which was not conducive to the aroma components of pine pollen.\[24,25\] Electron-beam treatment significantly reduced its content in pine pollen, while UHT made it increase. Isoborneol and thymemethylether were substances with high content in pine pollen and have a minty taste.\[26\] For isoborneol, EBI
Table 3. Volatile components from different treatments of pine pollen.

| Serial number | compounds                  | Content (µg/g) | Retention index |
|---------------|----------------------------|----------------|-----------------|
|               |                            | R-PP EBI-PP UHT-PP Calculated value | Reference value | Matching degree | Qualitative method |
| 1             | α-Pinene                   | 14.52 14.3 8.31 | 1012 1023        | 96               | MS/RI             |
| 2             | β-Pinene                   | 11.89 12.73 7.26 | 1094 1112        | 94               | MS/RI             |
| 3             | Sabine
| 4             | 2,4-(10)-Platyctadiene    | 0.74 0.74 0.71  | 1111            | 96               | MS/RI             |
| 5             | 3-Carene                   | 1.12 1.08 1.09 | 1128 1127        | 96               | MS/RI             |
| 6             | α-Phellandrene             | 0.47 0.49 0.17  | 1146 1163        | 94               | MS/RI             |
| 7             | β-Myrcene                  | 13.67 13.42    | 1150 1163        | 97               | MS/RI             |
| 8             | α – Terpinene              | 0.98 0.95 1.06  | 1159 1174        | 96               | MS/RI             |
| 9             | D-Limonene                 | 12.05 12.03 0.77 | 1180 1192        | 99               | MS/RI             |
| 10            | β-Phellandrene             | 32.87 31.39 21.32 | 1194 1201        | 94               | MS/RI             |
| 11            | γ-Terpinene                | 0.36           | 1227 1241        | 96               | MS/RI             |
| 12            | β – Rolene                 | 0.16           | 1237 1232        | 96               | MS/RI             |
| 13            | Terpinolene                | 1.66 1.75 1.03  | 1266 1274        | 98               | MS/RI             |
| 14            | 2,4-Dimethylstere
c
| 15            | Copae
| 16            | Longifolene                | 0.68 0.7 1.12  | 1557 1568        | 99               | MS/RI             |
| 17            | Caryophyllene              | 0.87 0.88 0.15  | 1591 1592        | 99               | MS/RI             |
| 18            | α-Murolene                 | 0.26           | 1723 1719        | 99               | MS/RI             |
| 19            | Calamene
| 20            | Caryene oxide              | 0.13 0.12 0.06  | 1992 1990        | 90               | MS/RI             |
| 21            | Camphene                   | 0.39 0.15      | 1054 1068        | 97               | MS/RI             |
| 22            | Cubebene                   | 0.29 0.3 0.09  | 1449 1459        | 97               | MS/RI             |
| 23            | Alcohols                    | 7.27 8.13 2.73 |                |                  |                   |
| 24            | hexanol                     | 0.58           | 1361 1362        | 83               | MS/RI             |
| 25            | 1-Heptanol                  | 0.18           | 1460 1457        | 83               | MS/RI             |
| 26            | Octanol                     | 1.38 1.39 0.48  | 1563 1558        | 90               | MS/RI             |
| 27            | Terpenes-4-ol              | 0.88 0.68 0.2  | 1600 1600        | 96               | MS/RI             |
| 28            | Pinocarveol                | 1.76 0.92 0.72  | 1658 1655        | 96               | MS/RI             |
| 29            | 1-Nonanol                   | 1.18 0.83      | 1664 1671        | 91               | MS/RI             |
| 30            | α-Terpineol                | 0.66 0.19      | 1697 1700        | 93               | MS/RI             |
| 31            | decanol                     | 1.09           | 1766 1767        | 91               | MS/RI             |
| 32            | Myrtenol                    | 0.96           | 1799 1794        | 95               | MS/RI             |
| 33            | Phenethyl alcohol           | 0.27 0.31      | 1922 1907        | 93               | MS/RI             |
| 34            | alcohol                     | 0.13           | 2113 2107        | 98               | MS/RI             |
| 35            | Cadinol                     | 0.18 0.19      | 2180 2167        | 91               | MS/RI             |
| 36            | Aldehydes                   | 3.93 3.91 5.15 |                |                  |                   |
| 37            | Hexanal                     | 0.78           | 1082 1081        | 90               | MS/RI             |
| 38            | Nonanal                     | 0.93 0.92 0.93  | 1391 1394        | 96               | MS/RI             |
| 39            | (E)-2-Octenal
| 40            | Campholenic aldehyde       | 0.46 0.45 0.1  | 1486            | 94               | MS               |
| 41            | Decanal                     | 1.01           | 1495 1494        | 91               | MS/RI             |
| 42            | Benzaldehyde                | 0.96 0.92 0.73  | 1522 1522        | 95               | MS/RI             |
| 43            | Myrtenol                    | 1.58 1.62 0.49  | 1628 1645        | 98               | MS/RI             |
| 44            | 2-Butyl-2-octenal           | 0.83           | 1667 1669        | 99               | MS/RI             |
| 45            | Ketones                     | 3.3 2.56 1.03  |                |                  |                   |
| 46            | Hexanoic acid
| 47            | Nonanoic Acid
| 48            | Octanoic acid
| 49            | Esters                      | 8.28 7.75 3.6  |                |                  |                   |
| 50            | Methyl octanoate            | 1.37 1.32 0.86  | 1388 1392        | 96               | MS/RI             |
| 51            | Methyl myristate            | 3.64 3.68 1.43  | 1579 1574        | 99               | MS/RI             |
| 52            | Methyl hexadecanoate        | 1.98 1.87 0.72  | 2221 2220        | 98               | MS/RI             |
| 53            | Methyl oleate               | 0.73 0.69 0.4  | 2451 2450        | 99               | MS/RI             |
| 54            | Others                      | 4.2 3.65 2.79  |                |                  |                   |

(Continued)
Sterilization had little effect on isoborneol content, and heat sterilization reduced isoborneol content by nearly half, but electron beam and UHT resulted in partial loss of thyme methyl ether, with heat sterilization losses as high as 1.01 µg/g.

Volatile aldehydes and organic acids were substances that produced lipid oxidation odor due to unsaturated bond breakage during the processing of pine pollen. The main reason for more aldehydes and organic acids produced by UHT-PP may be the oxidative cracking of unsaturated bonds in pine pollen due to high temperature, and on the other hand, it may be that UHT-PP has a longer processing time and is exposed to air for a longer period of time and is easily oxidized during the processing. The reason for the heavier lipid oxidation odor of UHT products may be that the volatile organic compounds were destroyed in the process of processing, in which the alkenes, alcohols and terpenes with fragrance were destroyed in the process of processing and the content was reduced, but the aldehydes and acids produced in the process were increased, which makes the aldehydes and acids account for more of the volatile substances, leading to the more prominent peculiar smell. Therefore, we can determine the degree of lipid oxidation by detecting acetaldehyde and acetic acid.

**Electron-beam and thermal sterilization on fatty acids in pine pollen**

As shown in Table 4, unsaturated fatty acids accounted for 70.65% of the total fatty acids in pine pollen. After Electron beam sterilization, the saturated fatty acid content in pine pollen was not significantly different compared with that before sterilization, and the total unsaturated fatty acid content decreased by only 4.70%. However, UHT had a significant effect on unsaturated fatty acids in pine pollen, resulting in the loss of nearly 29% of unsaturated fatty acids, while saturated fatty acids increased by 23.50% in the UHT-PP group.

The most abundant fatty acids measured in the pine pollen samples were palmitic acid (23.42–28.60%), oleic acids (34.8%–42.6%) and linoleic acids (15.9%–26.4%), which was in general agreement with that reported in the literature.

Total unsaturated acids (UFA) ranged from 61.1% to 68.7%, in which monounsaturated acids (MUFA) and polyunsaturated acids (PUFA) changed from 35.7% to 43.5% and 17.6% to 29.5%, respectively. High proportions of unsaturated acids, especially oleic and linoleic acids, were probably one of the most important factors influencing lipid peroxidation, resulting in the formation of aliphatic aldehydes, ketones, alcohols, furans and acids. Additionally, it should be pointed out that the presence of palmitic acid could improve stability against peroxidation; however, even a small amount of linoleic acid could have detrimental effects.

### Table 4

| Serial number | Compounds             | Content (µg/g) | Retention index |
|---------------|-----------------------|----------------|-----------------|
|               |                       | R-PP EBI-PP UHT-PP Calculated value | Reference value | Matching degree | Qualitative method |
| 54            | 2-Pentylfuran         | / 0.2 0.83    | 1220 1231       | 94               | MS/RI           |
| 55            | 0-isopropyltoluene    | 1.7 1.15 0.61 | 1255 1247       | 97               | MS/RI           |
| 56            | 2-Isopropyl-5-methylanisole | 1.57 0.86 0.56 | 1593 1574     | 95               | MS/RI           |
| 57            | DL-Isoborneol         | 2.46 2.44 1.32 | 1706 1708       | 92               | MS/RI           |
| 58            | 5-methyl-2-isopropylphenol | 0.17 0.15 0.08 | 2191 2198     | 87               | MS/RI           |
| **Total**     |                       | **121.31 119.19 61.15** |                  |                   |                 |

(UHT-PP: ultra-high temperature instantaneous sterilization sporoderm-broken pine pollen; EBI-PP: electron-beam irradiation sterilization sporoderm-broken pine pollen; R-PP: raw pine pollen)
During the UHT treatment and EBI of pine pollen, the greatest loss of linoleic acid and a smaller loss of oleic acid were observed, while medium-chain fatty acids such as palmitic acid, myristic acid and pentadecanoic acid increased during the heat treatment, with the greatest increase in palmitic acid content. According to the above gas chromatographic analysis, the odor of oil oxidation mainly consisted of volatile aldehydes and volatile acids, which were produced by the oxidative decomposition of unsaturated fatty acids during the processing of pine pollen. Therefore, we speculated that the unsaturated fatty acid that mainly causes the odor of pine pollen during processing was linoleic acid.\[4\]
The content of unsaturated fatty acids in UHT-PP was lower than in R-PP and EBI-PP, indicating that autoclaving led to the oxidation and even decomposition of unsaturated fatty acids, especially polyunsaturated fatty acids. The decrease in polyunsaturated fatty acid concentration also increased the monounsaturated fatty acid content of unsterilized pine pollen, UHT-PP and EBI-PP. The linoleic acid content decreased with the increase of oleic acid. These changes may further lead to acidification of pine pollen, which implies that UHT-processed pine pollen is susceptible to rancidity during actual storage.

**EBI sterilization on thiobarbituric acid value of pine pollen during storage**

As shown in Figure 7, R-PP had the highest rate, followed by UHT-PP, and EBI-PP had the lowest rate. The TBA values in the R-PP treatment group increased at the same rate over 90 days, eventually reaching 8 mg/100 g. After UHT sterilization, the TBA values reached 7.61 mg/100 g immediately at the beginning and then increased sharply to 11.50 mg/100 g. Although Electron beam sterilization increased the TBA value of treated pine pollen, it was only 4.71 mg/100 g at 90 days. thus, it can be seen that EBI sterilization has a good inhibitory effect on the oxidation of pine pollen during storage, while it has little effect on the quality of the sample within 90 days, which will delay the oxidation of fat, produce off-flavor and deterioration.

We analyzed the correlation between TBA values and enzyme activity, i.e., there was a correlation between thiobarbituric acid change values and enzyme activity during storage. In conclusion, lipid oxidation of pine pollen caused by enzymatic reactions is the main role during storage, while oxidation of pine pollen caused by other non-enzymatic factors such as oxygen is a secondary role.

**Conclusion**

The content of oxidation products was significantly higher in UHT-PP than in EBI-PP. The loss of terpenes and alcohols and the production of aldehydes in the aromatic odor were lower compared to UHT. Meanwhile, EBI sterilization significantly reduced the LOX activity of plant endogenous lipoxygenase, which had little effect on the nutritional composition and sensory quality of pine pollen. In storage tests, the quality of EBI-PP was more stable, indicating that EBI sterilization is more beneficial to the processing and storage of pine pollen than heat sterilization. The lipid oxidation odor from the oxidation of unsaturated fatty acids in pollen was mainly caused by volatile aldehydes and volatile acids. EBI sterilization has less effect on the odor of pine pollen and can maintain the integrity of its main aroma components. Volatile aldehydes (e.g. acetaldehyde) and volatile acids (e.g. acetic acid) can be used as indicators for lipid oxidation detection. EBI sterilization technology has a low degree of damage to the lipids of pine pollen, while ultra-high temperature sterilization technology causes a large amount of unsaturated fatty acid loss during the processing. Lipid oxidation odor substances are produced by the oxidation of unsaturated fatty acids, and the main unsaturated fatty acid causing lipid oxidation odor is linoleic acid. Protective treatment of linoleic acid during processing can reduce the lipid oxidation odor generated during processing.

In order to avoid lipid oxidation of pine pollen, the heat should be reduced and the time of exposure to air should be shortened. During the processing of pine pollen, too much reducing substances such as polyphenols and vitamin C are lost, making pine pollen more susceptible to oxidation. Meanwhile, heating tends to activate lipoxygenase in pine pollen, leading to the loss of unsaturated fatty acids in pine pollen, and more loss of linoleic acid in unsaturated fatty acids, resulting in more volatile aldehydes and volatile acids. In the storage process, enzyme reaction plays a major role, so enzyme activity as an important storage indicator, sterilization process should be as low as possible to reduce enzyme activity.

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