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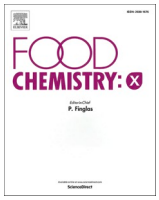

# Formation of lipid-derived volatile products through lipoxygenase (LOX)- and hydroperoxide lyase (HPL)- mediated pathway in oat, barley and soy bean

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ABSTRACT

The aim of this study was to explore the formation of volatile lipid oxidation products by the lipoxygenase (LOX)-hydroperoxide lyase (HPL)-mediated pathway in oat, barley and soy bean. LOX activity was found only in barley and soy bean samples, but the lipase and HPL activity was detected in all samples. HPL showed particularly high activity with 13-hydroperoxides, while the activity was quite low when using 9-hydroperoxides, especially in the oat and barley. The optimum pH for HPL in different samples was similar, i.e., pH 6–7. In this condition, the volatile compounds formed dramatically with aldehydes and furans as the dominant products. Furthermore, a remarkable enzymatic degradation of lipids occurred during the preparation of food models with highly refined rapeseed oil (RO) and rapeseed oil fatty acid (ROFA) emulsions, where the ROFAs were more prone to oxidation than RO. This study shows the significance of lipid-degrading enzymes in plant-food flavour formation.

## 1. Introduction

It is estimated that the global population will reach 9.8 billion by 2050, which necessitates increased agricultural production to meet the increasing demand for food. Plant-based foods are attracting attention as a way to meet this challenge (Wang et al., 2022; Webb, Li, & Alavi, 2023). Cereals and legumes are the main sources of protein in the human diet, and the physicochemical functionalities and nutritional quality of cereals and legumes have been well studied. Oat and barley are important cereal crops that are widely cultivated worldwide. They provide good sources of human food because of their unique taste, high nutritional quality and good structure for preparing food products (Kamal et al., 2022; Obadi, Sun, & Xu, 2021). Due to the high content of dietary fibres (such as β-glucan), tocopherols and avenanthramides, oat and barley play important roles in the prevention of cardiovascular disease and cancer (Obadi et al., 2021; Rasane, Jha, Sabikhi, Kumar, & Unnikrishnan, 2015). Another staple legume that is cultivated and consumed worldwide is soy bean, which is rich in protein content and bioactive peptides and also plays a role in preventing cardiovascular disease (Wang, He, & Raghavan, 2023). However, food loss and waste impact up to one-third of global food production, including approximately 30% of cereals and 45% of fruits and vegetables (Kah, Tufenkji, & White, 2019). One crucial contributor to this loss is the formation of undesirable off-flavours, resulting in the deterioration of the quality of cereal and legume products.

Many factors can bring about changes in the flavour quality of plant-based foods, including lipid-degrading enzymes, storage conditions and processing methods (Cai et al., 2021; Wang, Cui, Wang, Li, & Qiu, 2021). Lipid degradation through the action of lipid-degrading enzymes has a critical impact on food flavours and can be the cause of undesirable off-flavours. Oat, barley and soy bean contain lipids, as well as lipid-degrading enzymes, such as lipase, lipoxygenase (LOX) and peroxidase, which provides the ideal prerequisite for lipid degradation and the formation of off-flavours, especially when the grains are broken or milled into flours (Tang et al., 2023; Yang, Zhou, Xing, Guo, & Zhu, 2022).

Lipase is widely present in cereals and legumes, including oat, barley (Khasi & Azizkhani, 2022) and black soy bean (Yang et al., 2022). It is

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the first enzyme involved in lipid degradation through the action of hydrolysing triglycerides (TAGs) to release free fatty acids (FFAs). LOX catalyses the oxidation of polyunsaturated fatty acids (PUFAs) containing (*Z*),(*Z*)-1,4-pentadiene moiety (e.g. linoleic acid and linolenic acid) to generate lipid hydroperoxides, which are quite unstable and may easily degrade into secondary volatile and nonvolatile lipid oxidation products (Yang et al., 2023). The effect of LOX in soy bean has been suggested to be associated with the production of an unpleasant, beany flavour (Patel, Kumar, Priyadarshini, Singla, & Sandhu, 2023). LOX activity varies in cereals, as its activity was found to be fairly high in barley but rather low in oat (Tang et al., 2023). Legumes, such as black soy bean and faba bean, are known to possess high LOX activity (Lampi, Yang, Mustonen, & Piironen, 2020; Yang et al., 2022).

The degradation of hydroperoxides through the LOX pathway involves many of lipid-degrading enzymes, including hydroperoxide lyase (HPL), hydroperoxide dehydrase and peroxygenase (Yang et al., 2023). HPL is a downstream enzyme of the LOX-pathway in lipid oxidation and belongs to the CYP74 family, which can catalyse the C—C bond cleavage of lipid hydroperoxides to produce volatile aldehydes and various oxygen-containing acids (Stolterfoht, Rinnofner, Winkler, & Pichler, 2019). Depending on the specificities of the substrate, HPL can be categorised into three types: 9-HPL, 13-HPL and 9/13-HPL. The 9-HPL specifically cleaves to the 9-position hydroperoxide, while 13-HPL specifically cleaves to the 13-position hydroperoxide and 9/13-HPL has the activity of both. The main volatile compounds produced (C6 aldehyde and C9 aldehyde) by HPL are important signal molecules in plants that regulate stress responses and are also the main components of the aromatic odour of fruits and vegetables (Wu, 2023; Yue et al., 2022). However, compared to LOX, the role of HPL in creating flavour compounds in cereal and legumes has been less studied, even though the volatile compounds produced by HPL have a significant impact on food quality and, in particular, on the deterioration of food flavour (McGorrin, 2019).

Several recent studies have focused on characterising the activity of HPL in different plants and the effect of enzyme-mediated volatile substances on plant physiology (Brosset & Blande, 2022; Stolterfoht et al., 2019). HPL has been found in plants such as tomato, *arabidopsis thaliana* and pepper (Magalhães, Filho, Garruti, Massaretto, & Purgatto, 2021; Rodrigues Magalhaes et al., 2023; Stolterfoht et al., 2019) and has also been detected in some cereals, including barley and rice (Chehab et al., 2006; Koeduka, Stumpe, Matsui, Kajiwara, & Feussner, 2003). However, little is known about the pathway and the impact of volatile lipid oxidation products mediated by HPL on flavour quality in cereals and legumes, including oat, barley and soy bean. Many studies have investigated the formation of lipid-degradation products in oat (Yang et al., 2023), rice (Yuan et al., 2019) and faba bean (Lampi et al., 2020) and have found that a large number of flavour compounds, such as aldehydes and furans, were produced, which was related to lipase activity and the LOX degradation pathway. These studies proposed that HPL has great impacts that are responsible for undesirable off-flavours. Thus, it is necessary to clarify the role of the LOX-HPL-mediated pathway in the formation of volatile compounds in cereals and legumes to understand the mechanism of off-flavours and to provide a theoretical basis for recommendations regarding the storage of cereal foods.

Thus, our overall aim was to study the LOX-HPL-mediated formation of lipid-derived volatile products in oat, barley and soy bean, since they are used as promising food ingredients due to their great potential for developing high quality of food products. The special focus was on the catalytic characteristics of HPL. Furthermore, lipid degradation reaction at different pH conditions during food processing was also investigated using food models. The results of this study can provide knowledge about the LOX-HPL-mediated pathway and the mechanism for the formation of lipid oxidation compounds in plant foods, as well as lay a theoretical foundation for the development of the food industry.

## 2. Materials and methods

### 2.1. Oat, barley and soy bean samples

Dehulled oats from different regions and cultivars were used in this study. The Qinghai oat (OQH) sample was provided by the Qingmai Food Co., Ltd. (Qinghai, China), and another oat sample (ONMG) was provided by the Inner Mongolia Oatshouse Ecological Agriculture Development (Group) Co., Ltd. (Inner Mongolia, China). Dehulled barley samples, including white barley (WB) and blue barley (BB), were purchased from the Xinning Biotechnology Co., Ltd. (Qinghai, China). Soy bean samples were purchased from the Shanghai Freshippo Internet of Things Co., Ltd. (Heilongjiang, China).

All the grain samples were milled using a high-power pulveriser (Huangdai, SUS 304–800 Y) and then passed through a 60-mesh sieve. The milled flours were stored at −20 °C before analysis.

### 2.2. Chemicals and reagents

Linoleic acid (purity >99%), α-linolenic acid (purity >99%), 4-nitrophenyl butyrate (*p*-NPB; purity >98%) and 4-nitrophenyl palmitate (*p*-NPP; purity >98%) were purchased from the Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Lipoxygenase (LOX-1) from *glycine* max (soy bean) was supplied by Sigma Alderich (St. Louis, USA). Dimethyl sulfoxide (DMSO) was purchased from the Macklin Biochemical Co., Ltd. (Shanghai, China). Rapeseed oil (RO) was purchased from the Yihai Kerry Arawana Holdings Co., Ltd. (Shanghai, China). All the other chemical reagents were of analytical grade.

### 2.3. Determination of lipase activity and substrate specificity

To determine the lipase activity and substrate specificity of oat, barley and soy bean samples, a simple method by *using* was used for measurement (Lampi et al., 2020). Briefly, 1 g of *p*-NPB was dissolved in a small amount of DMSO and diluted to 50 mL to obtain 100 mM of concentrated substrate solution. The *p*-NPP substrate was prepared in the same way. To determine lipase activity, 100 mM of the concentrated substrate solution was diluted to 2 mM with a buffer solution (50 mM of potassium phosphate buffer containing 0.1% Triton X-100, pH 8).

For lipase extraction, 10 mL of distilled water was added to 2 g of oat and soy bean flour and to 4 g of barley flour. Then, the mixture was vortexed using a vortex device and left standing for 30 min at room temperature. After that, the slurry was centrifuged at 9000*g* for 10 min, and the supernatant was collected and used as the crude enzyme extract. For lipase activity measurement, an aliquot of 100 μL, 200 μL and 25 μL of oat, barley and soy bean crude enzyme solution, respectively, was mixed with 2 mM of substrate solution to a final volume of 1 mL in a cuvette. A UV–visible spectrophotometer (Unico UV-2802, USA) was used to measure the changes in absorbance within 180 s at 405 nm. Lipase activity was calculated using a molar extinction coefficient of $\varepsilon = 16.05\ mM^{-1}\ cm^{-1}$ and shown as $\mu mol\ min^{-1}\ g^{-1}$ flour. Each sample was measured three times.

### 2.4. Determination of LOX activity and substrate specificity

LOX activity in the oat, barley and soy bean samples was measured using linoleic acid and α-linolenic acid as substrates (Tang et al., 2023). The substrate solution (10 mM) was prepared using 0.14 g of either linoleic acid or α-linolenic acid and mixed with the same amount of Tween 20 and 10 mL of distilled water, and then 600 μL of 1 M NaOH was added and a final volume of 50 mL was achieved by adding distilled water. The LOX crude enzyme extract was prepared following the procedure described in Section 2.3, except that 4 g of oat flour, 4 g of barley flour and 0.05 g of soy bean flour, respectively, were mixed with 10 mL of distilled water.

LOX activity was measured following the procedure set out by Tang

et al. (2023). Briefly, the reaction system contained 200 μL of each substrate solution in a 0.1 M potassium phosphate buffer (pH 6), except that 800 μL of oat crude enzyme extract, 50 μL of barley crude enzyme extract and 10 μL of soy bean crude enzyme extract were used. The reaction was conducted in a water bath at 30 °C for 3 min and terminated by adding 1 mL of 0.3 M KOH. Absorbance was measured at 234 nm using a UV visible spectrophotometer (Unico UV-2802, USA). LOX activity was expressed as μmol $min^{-1}$ $g^{-1}$ flour, and the molar extinction coefficient of ε = 26,000 L $mol^{-1}$ $cm^{-1}$ was used for calculation. Each sample was measured three times.

### 2.5. Determination of HPL activity and substrate specificity

#### 2.5.1. Preparation of fatty acid hydroperoxides

Soy bean LOX-1 specifically produces 13-hydroperoxides at pH 8.5–9, and 9-hydropoxides at pH 6.5 (Schaich, Shahidi, Zhong, & Eskin, 2013). Linoleic acid and α-linolenic acid were used to react with soy bean LOX-1 for the production of hydroperoxides, following the method described by Gargouri, Drouet, and Legoy (2004) with some modifications. To produce 13-hydroperoxy octadecadienoic acid (13-HPOD) and 13-hydroperoxy octadecatrienoic acid (13-HPOT), about 100 mg of linoleic acid or α-linolenic acid was added to 100 mL of 0.2 M borate buffer at pH 9 pre-aerated with oxygen to saturation, and then 3 mg of soy bean LOX-1 dissolved in a small amount of borate solution was added. After that, the mixture was stirred using magnetic stirring, and the reaction was terminated when the absorbance of the reactant remained unchanged at 234 nm that was considered a completion for hydroperoxide production. The same method was used to produce the 9-hydroperoxy octadecadienoic acid (9-HPOD) and 9-hydroperoxy octadecatrienoic acid (9-HPOT) compounds, except that the reaction condition was changed to pH 6.5. For each experiment, the reaction liquid was extracted twice with an equal volume of anhydrous ether, and then the organic phase was combined, dried with anhydrous magnesium sulfate and evaporated by rotary evaporation at 30 °C. Finally, the residue containing fatty acid hydroperoxide was redissolved in ethanol to a final concentration of 4 mM of substrate solution for the following HPL activity measurement.

#### 2.5.2. Determination of HPL activity and optimal pH

The HPL activity in oat, barley and soy bean was measured following the method reported by Feng et al. (2022). The method for extracting the HPL enzyme was the same as described in Section 2.3, except that 1 g of each flour sample was mixed with 6 mL of distilled water. To measure HPL activity, 40 μL of the enzyme solution was mixed with 10 μL of each substrate solution (4 mM), while 8 μL of soy bean enzyme extract was mixed with 2 μL of substrate solution, and buffers with different pH values (50 mM, pH 4–9) were added to a final volume of 3 mL (i.e. acetate buffer at pH 4.0–5.5, phosphate buffer at pH 6.0–7.0, Tris-HCl buffer at pH 7.5–8.5 and borate buffer at pH 9.0). A UV–visible spectrophotometer (Unico UV-2802, USA) was used to record changes in absorbance within 180 s at 234 nm. The molar extinction coefficient of ε = 25,000 L $mol^{-1}$ $cm^{-1}$ was used for calculation. HPL activity was reported as μmol $min^{-1}$ $g^{-1}$ flour. Each sample was measured three times.

### 2.6. Determination of HPL substrate specificity through the formation of volatile lipid oxidation compounds

To characterise the substrate specificity of HPL in oat, barley and soy bean, the differently produced hydroperoxides described in Section 2.5.1 (i.e. 9-HPOD, 9-HPOT, 13-HPOD and 13-HPOT) were used as substrates. The reaction system was conducted similarly to that described in Section 2.5.2, but the reaction was done at pH 6.5 (50 mM phosphate buffer) in a 10 mL SPME vial and reacted in a 25 °C water bath for 10 min. Finally, the reaction was terminated by adjusting the pH to below 3 using HCl solution. Volatile lipid oxidation products were detected using a headspace solid-phase microextraction gas chromatography-mass spectrometer (HS-SPME-GC–MS) (GC System 7890 A-5975C, Agilent Technologies, USA) (Tang et al., 2023). The solid-phase microextraction fibre used in this experiment was polydimethylsiloxane/divinylbenzene/carboxen (PDMS/DVB/CAR, 50/30 μm), and the column was equipped with DB-WAX (30 m × 250 μm × 0.25 μm) (Agilent Technologies, California, USA). The volatile compounds were extracted in a headspace injector oven at 250 rpm for 30 min. The initial oven temperature of 60 °C was maintained for 4 min, and then the temperature was increased at a rate of 5 °C/min to 90 °C. After that, the temperature increased by 10 °C/min to the final temperature of 240 °C, where it was held for 8 min. The amount of volatile compounds was quantified by peak areas (counts * s * $10^6$). Each sample was analysed three times. The linear retention indices (LRI) were calculated based on the retention times of a homologous series of *n*-alkanes. The identification of volatile compounds was performed by matching their total ion mass spectra with the database NIST (Version 2.0, Gaithersburg, MD, USA) and by comparing the LRI to the NIST Chemistry WebBook (https://webbook.nist.gov/chemistry/name-ser/).

### 2.7. Potential for the LOX-HPL-mediated formation of lipid degradation products in food models

Finally, the potential for the LOX-HPL-mediated formation of lipid oxidation products in oat, barley and soy bean food models was studied at different pHs and with different lipid types, and the actions of lipase and LOX during food model preparation were evaluated. The food models used the sample flour, highly refined rapeseed oil (RO) and rapeseed oil fatty acids (ROFAs) to prepare emulsions according to the method reported by Lampi et al. (2020). To prepare the ROFAs, the RO (450 mg) was mixed with 1 mL of saturated KOH and 16 mL of absolute ethanol and then saponified in an 85 °C water bath for 30 min. Then, 20 mL of water and the same amount of organic solvent (diethyl ether and heptane 1:1) were added to extract the saponified RO; then, the aqueous phase was collected, and the pH was adjusted to 4. The same organic phase was added again for further extraction and then collected. Finally, the organic phase was dried with nitrogen and dissolved in 10 mL of heptane.

To prepare the food models, 2.5 g of each sample flour was mixed with 15 mL of buffer at different pHs (pH 5, 6.5 and 8) and homogenized evenly. The mixtures were allowed to stand for 30 min and then centrifuged (4 °C, 9000*g*, 10 min) to collect the supernatant. The 15 mL of supernatant was mixed with 450 mg of RO or ROFA (3% *w/v*) and homogenized at 8000 rpm for 2 min. The homogenized sample was placed in a water bath (25 °C) for 10 min, and the pH was adjusted to <3 using 6 mol/L of HCl to end the reaction. Finally, the volatile compounds produced in the food models were determined by HS-SPME-GC–MS, using the same method as described in Section 2.6. The amount of volatile compounds produced in each sample was quantified by peak area (counts * s * $10^6$). Each sample was analysed three times.

### 2.8. Statistical analysis

All data were expressed as mean values ± standard deviations. Statistical analysis was performed by one-way analysis of variance (ANOVA) and then by Tukey's test using SPSS software version 26.0 (IBM SPSS Statistics, Chicago, IL, USA). A value of $p < 0.05$ indicated statistical significance. Origin 2021 software (Origin Lab Corporation, Northampton, MA, USA) was used to create the figures. Multivariate analysis of the volatile data matrices was conducted using the online platform Metaboanalyst (https://www.metaboanalyst.ca/), and the data were pretreated through square root transformation and auto scaling to achieve, respectively, standardisation and normalisation.

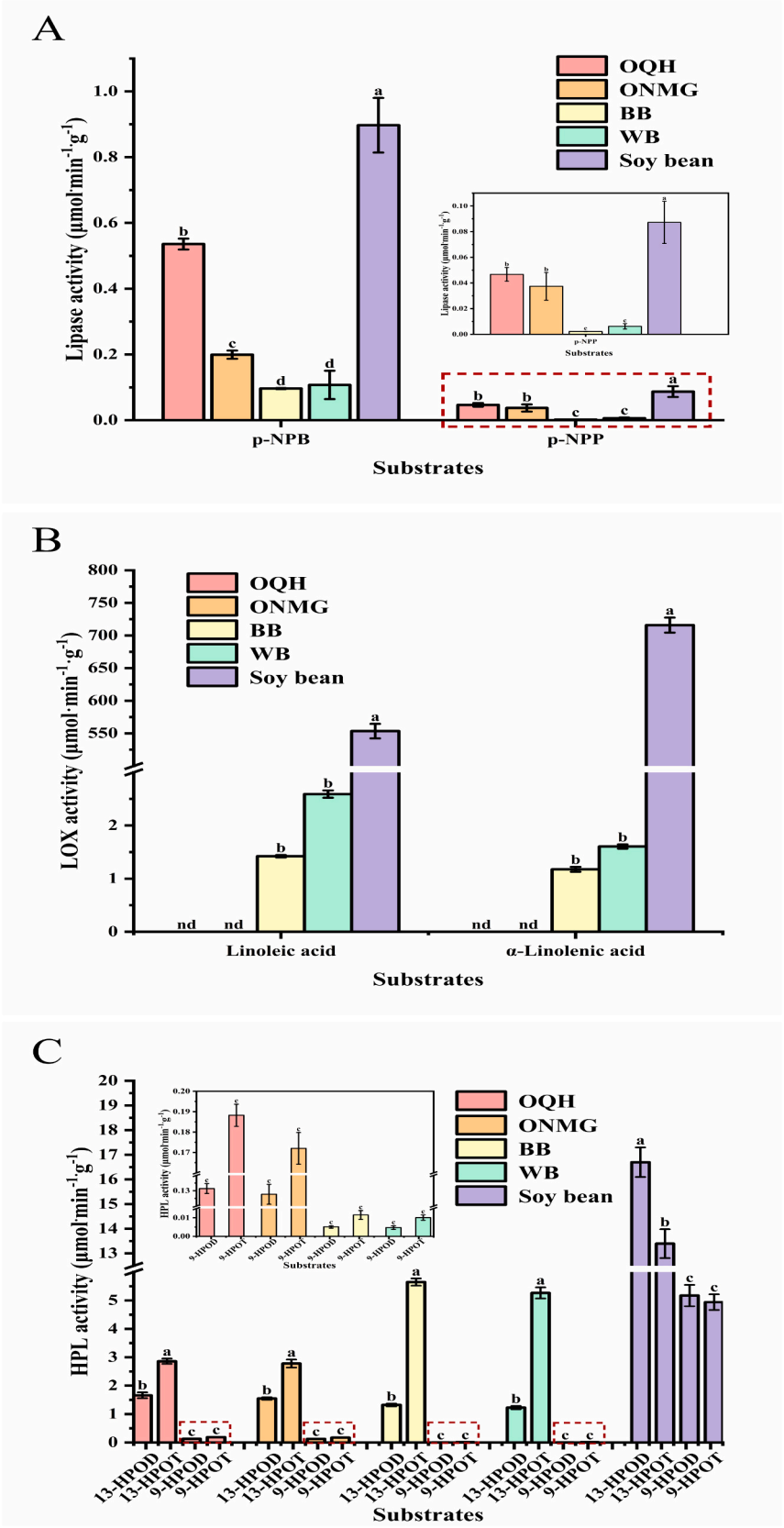


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**Fig. 1.** The activity and substrate specificity of lipase, lipoxygenase (LOX) and hydroperoxide lyase (HPL) in oat, barley and soy bean using (A) 4-nitrophenyl butyrate (*p*-NPB) and 4-nitrophenyl palmitate (*p*-NPP), (B) linoleic acid and α-linolenic acid, and (C) 13-hydroperoxy octadecadienoic acid (13-HPOD), 13-hydroperoxy octadecatrienoic acid (13-HPOT), 9-hydroperoxy octadecadienoic acid (9-HPOD) and 9-hydroperoxy octadecatrienoic acid (9-HPOT). For samples, OQH = the Qinghai oat; ONMG = the Inner Mongolia oat; WB = the white barley; and BB = the blue barley. [a–d] The letters a–d show significant differences within each treatment condition, as estimated by Tukey's test ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

## 3. Results and discussion

### 3.1. Activity and substrate specificity of lipase in oat, barley and soy bean

As shown in Fig. 1A, the oat cultivars (OQH, 0.54 ± 0.02 μmol $min^{-1}$ $g^{-1}$ flour; ONMG, 0.20 ± 0.01 μmol $min^{-1}$ $g^{-1}$ flour) showed a higher lipase activity than the barley samples (BB, 0.10 ± 0.002 μmol $min^{-1}$ $g^{-1}$ flour; WB, 0.11 ± 0.04 μmol $min^{-1}$ $g^{-1}$ flour), but the lipase activity in soy bean (0.90 ± 0.08 μmol $min^{-1}$ $g^{-1}$ flour) was the highest when using *p*-NPB as a substrate. This is consistent with the results of Yang, Piironen, and Lampi (2017), who reported that the lipase activity in faba bean was higher than that in oat. This may indicate that legumes generally show higher lipase activity than cereals.

A short-chain substrate of *p*-NPB and a long-chain substrate of *p*-NPP were used to measure lipase activity because it would be interesting to know the selectivity of lipases, as also used by Lampi et al. (2020). When comparing the lipase activity of the two substrates, it was seen that the lipase activity of all samples was much higher (ca. 10-fold) when using *p*-NPB than when using *p*-NPP (Fig. 1A). Lampi et al. (2020) found that the activity of *p*-NPP as a substrate was only one-fifteenth that of *p*-NPB, partly because the short acyl group can also be hydrolysed by nonspecific esterase (Gilham & Lehner, 2005) in cereals, such as oat and barley, and in legumes, such as soy bean. In addition, both oat and faba bean lipases may be able to catalyse triacylglycerols (e.g. triolein and trilinolein) and RO (Lampi et al., 2020), showing the potential of lipid hydrolysis during food processing.

### 3.2. Activity and substrate specificity of LOX in oat, barley and soy bean

Linoleic acid and α-linolenic acid were used to characterise the LOX activity in oat, barley and soy bean. As shown in Fig. 1B, LOX activity was observed only in barley and soy bean, but not in oat. Similarly,

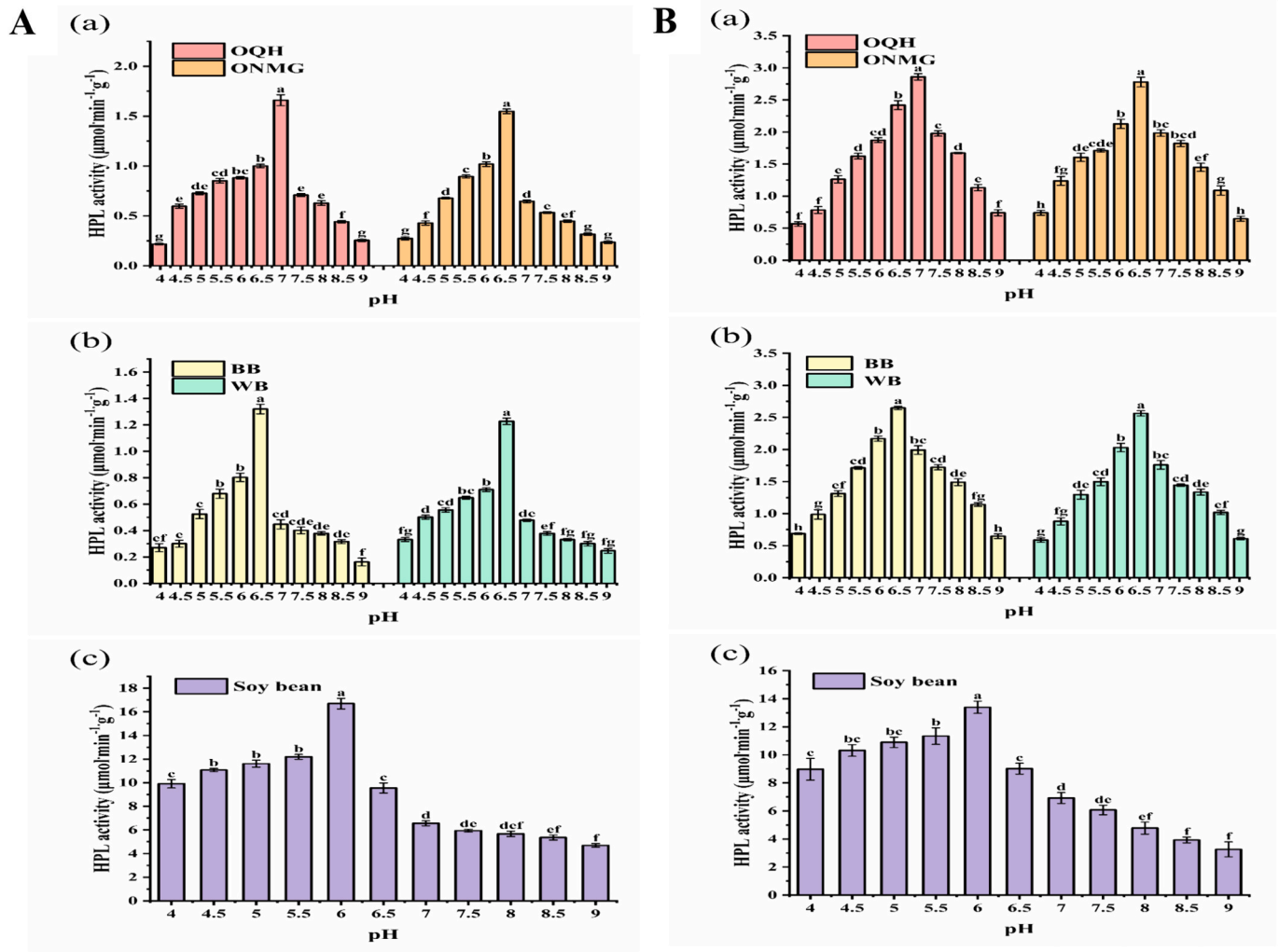


**Fig. 2.** Optimal pH of hydroperoxide lyase (HPL) activity in oat, barley and soy bean using the substrates of: (A) 13-HPOD, (B) 13-HPOT, (C) 9-HPOD and (D) 9-HPOT, where the sub-figures a-c indicated the HPL activity in (a) oat, (b) barley and (c) soy bean. For samples, OQH = the Qinghai oat; ONMG = the Inner Mongolia oat; WB = the white barley; and BB = the blue barley. [a–h] The letters a–h indicate significant differences within each treatment condition, as estimated by Tukey's test ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

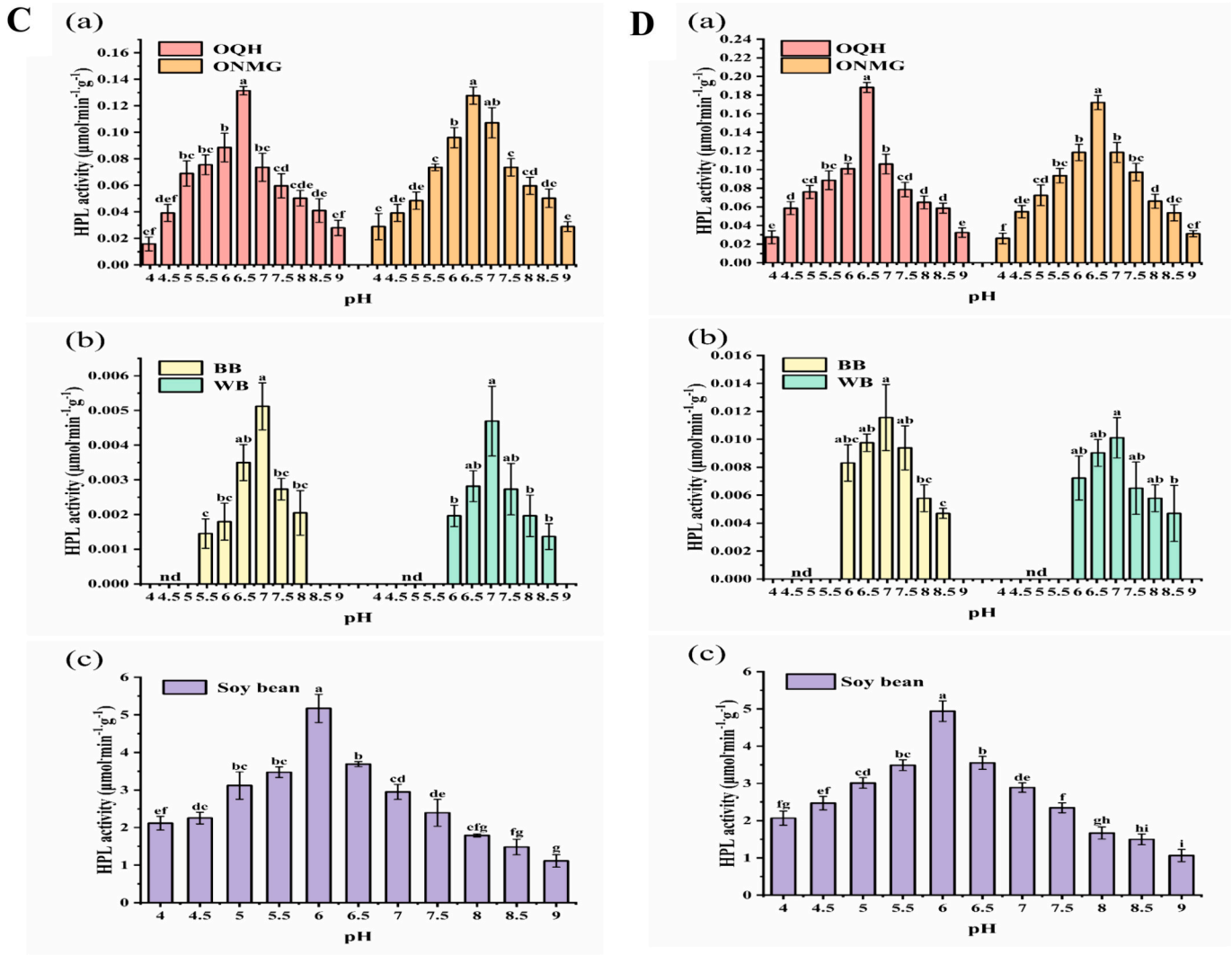


**Fig. 2.** (*continued*).

previous studies have not found LOX activity in oat (Tang et al., 2023; Yang et al., 2017). It was seen that the LOX activity in soy bean was dramatically higher than it was in barley. The LOX activity ranged from 1.17 ± 0.05–1.43 ± 0.02 μmol $min^{-1}$ $g^{-1}$ flour and 1.60 ± 0.04–2.59 ± 0.07 μmol $min^{-1}$ $g^{-1}$ flour in BB and WB, respectively, and the activity in soy bean was 553.50 ± 11.12–715.90 ± 11.56 μmol $min^{-1}$ $g^{-1}$ flour. High LOX activity has also been found in soy bean (375.8 μmol $min^{-1}$ $g^{-1}$) (Feng et al., 2022) and faba bean (ca. 350 μmol $min^{-1}$ $g^{-1}$) (Lampi et al., 2020), showing a high risk of LOX-induced enzymatic lipid oxidation when soy bean ingredients are included in foods. Although LOX was low in barley and oat, its activity should not be ignored during long-term storage.

For the LOX substrate specificity, barley LOX was able to oxidise both linoleic acid and α-linolenic acid, but we were not able to differentiate them due to the fairly equal activity values. However, for the soy bean LOX, α-linolenic acid seemed to be preferable as a substrate (Fig. 1B). This may be due to the difference in the LOX isomerases of barley and soy bean, where soy bean contains three types of LOXs that are able to catalyse both the 9- and 13-positions of α-linolenic acid in this pH condition (pH 6) (Schaich et al., 2013). Further studies are needed to learn more about the LOX properties of cereals, including barley.

### 3.3. Characterisation of hydroperoxide lyase in oat, barley and soy bean

To study the characteristics of HPL in oat and barley, the optimum pH for each sample was determined using four substrates: 13-HPOD, 13-HPOT, 9-HPOD and 9-HPOT. As shown in Fig. 2, the optimal pH of HPL in oat, barley and soy bean samples was generally between pH 6.0 and 7.0. Previous studies have reported that the optimum pH of HPL in mung bean seedlings was 6.5, and the optimum pH in sugar beet leaves was 6.7 (Rabetafika et al., 2008; Rehbock, Ganβer, & Berger, 1998). However, evidence about the response of HPL to different pHs and to different cereals and legumes is lacking.

The four substrates (13-HPOD, 13-HPOT, 9-HPOD and 9-HPOT) were used to measure HPL activity under the optimum pH conditions for each sample (Fig. 1C). It was seen that HPL activity was significantly different ($p < 0.05$) with the different substrates. In general, when using the 13-HPOD substrate, HPL activity in oat and barley was similar (ca. 1.5 μmol $min^{-1}$ $g^{-1}$ flour), and the values were slightly higher when using the 13-HPOT substrate. However, HPL activity was quite low when 9-HPOD and 9-HPOT were used for measurement, especially in the oat and barley samples (Fig. 1C). Although soy bean had a much higher HPL activity, the activity when using 13-HPOD was two to three times greater than when using 9-HPOD and 9-HPOT. This may indicate that oat and barley contain HPL isomerases, with 13-HPL dominant and lower 9-HPL activity. However, little is known about the activity and

**Table 1**
Formation of the identified volatile lipid oxidation compounds in oat, barley and soy bean using four types of hydroperoxide substrates (counts * s * $10^6$) [a].

| Volatile compounds [b] | 13-HPOD | | | 13-HPOT | | | 9-HPOD | | | 9-HPOT | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Oat | Barley | Soy bean | Oat | Barley | Soy bean | Oat | Barley | Soy bean | Oat | Barley | Soy bean |
| *Aldehydes* | | | | | | | | | | | | |
| Hexanal | 27.85 ± 0.77 | 33.6 ± 0.58 | 435.58 ± 3.52 | 9.38 ± 0.11 | 3.08 ± 0.12 | 193.79 ± 3.57 | 4.35 ± 0.10 | 4.60 ± 0.09 | 57.80 ± 0.24 | 2.96 ± 0.16 | 1.48 ± 0.02 | 25.91 ± 1.98 |
| (*E*)-2-Hexenal | nd | nd | 3.70 ± 0.14 | 3.41 ± 0.12 | 3.30 ± 0.09 | 141.14 ± 3.00 | nd | nd | 0.32 ± 0.01 | 3.15 ± 0.07 | 3.40 ± 0.06 | 26.88 ± 0.34 |
| Octanal | 0.55 ± 0.01 | 0.40 ± 0.02 | 5.08 ± 0.07 | nd | nd | nd | nd | 0.10 ± 0.00 | nd | 1.21 ± 0.04 | 0.29 ± 0.01 | 2.57 ± 0.09 |
| (*E*)-2-Heptenal | 0.58 ± 0.01 | 0.51 ± 0.02 | 6.59 ± 0.15 | 0.29 ± 0.00 | nd | nd | 0.13 ± 0.01 | 0.15 ± 0.01 | 1.02 ± 0.02 | 0.39 ± 0.01 | nd | 2.56 ± 0.14 |
| 1-Nonanal | 3.53 ± 0.04 | 4.07 ± 0.24 | 40.30 ± 2.30 | 3.67 ± 0.11 | 2.82 ± 0.09 | 20.06 ± 0.96 | nd | nd | nd | 5.28 ± 0.18 | 2.46 ± 0.11 | 6.17 ± 0.20 |
| (*Z*)-2-Octenal | 2.12 ± 0.07 | 1.83 ± 0.05 | 24.87 ± 0.14 | nd | nd | 20.84 ± 0.15 | 2.05 ± 0.09 | 1.90 ± 0.11 | 6.49 ± 0.26 | 0.56 ± 0.03 | 0.16 ± 0.01 | 9.91 ± 0.60 |
| (*E,E*)-2,4-Heptadienal | nd | nd | nd | 0.56 ± 0.00 | 0.21 ± 0.01 | 1.27 ± 0.09 | nd | nd | nd | 0.21 ± 0.01 | 0.23 ± 0.01 | 0.34 ± 0.01 |
| Decanal | nd | nd | nd | 0.56 ± 0.02 | nd | nd | nd | nd | nd | nd | nd | nd |
| (*E*)-2-Nonenal | 1.53 ± 0.09 | 1.69 ± 013 | 14.01 ± 0.78 | 1.06 ± 0.05 | 0.25 ± 0.02 | 7.80 ± 0.14 | 0.21 ± 0.00 | 0.21 ± 0.00 | 1.00 ± 0.03 | 1.19 ± 0.09 | nd | 3.68 ± 0.15 |
| (*E,E*)-2,4-Nonadienal | 1.16 ± 0.07 | 0.79 ± 0.01 | 3.77 ± 0.06 | 0.50 ± 0.02 | nd | nd | 0.12 ± 0.00 | 0.11 ± 0.00 | 0.48 ± 0.02 | 0.24 ± 0.01 | nd | nd |
| (*E,E*)-2,4-Decadienal | 0.88 ± 0.02 | 0.38 ± 0.01 | 3.35 ± 0.09 | 1.29 ± 0.04 | nd | 3.12 ± 0.14 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.14 ± 0.01 | 0.15 ± 0.02 | nd | 0.51 ± 0.04 |
| *Ketones* | | | | | | | | | | | | |
| 2-Nonanone | nd | nd | nd | 0.42 ± 0.01 | nd | nd | nd | nd | nd | 0.03 ± 0.00 | nd | nd |
| 3-Octen-2-one | nd | nd | nd | nd | nd | nd | 0.09 ± 0.00 | 0.09 ± 0.00 | 0.35 ± 0.01 | nd | nd | nd |
| 3,5-Octadien-2-one | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.06 ± 0.00 | 0.06 ± 0.00 | nd |
| *Alcohols* | | | | | | | | | | | | |
| 1-Penten-3-ol | nd | nd | nd | 2.38 ± 0.07 | nd | nd | nd | nd | nd | nd | nd | nd |
| 1-Pentanol | 0.57 ± 0.02 | 0.59 ± 0.02 | 1.79 ± 0.05 | 0.26 ± 0.01 | nd | 1.42 ± 0.09 | 0.39 ± 0.01 | 0.39 ± 0.01 | 0.86 ± 0.04 | nd | nd | 0.45 ± 0.03 |
| 2-Penten-1-ol | nd | nd | nd | 1.23 ± 0.10 | 0.98 ± 0.02 | 4.48 ± 0.19 | nd | nd | nd | 0.49 ± 0.06 | 0.43 ± 0.01 | nd |
| 1-Octen-3-ol | 1.35 ± 0.06 | 1.23 ± 0.12 | 14.18 ± 0.44 | nd | nd | 15.30 ± 0.46 | 0.10 ± 0.00 | nd | 2.37 ± 0.13 | 0.17 ± 0.01 | nd | 5.83 ± 0.15 |
| 1-Octanol | 0.25 ± 0.01 | 0.21 ± 0.01 | 1.41 ± 0.12 | 0.16 ± 0.01 | 0.18 ± 0.01 | 0.98 ± 0.04 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.10 ± 0.00 | nd | nd | 1.57 ± 0.17 |
| n-Pentadecanol | 0.96 ± 0.03 | 1.14 ± 0.03 | 1.99 ± 0.10 | 0.59 ± 0.01 | 0.71 ± 0.02 | 3.61 ± 0.15 | 0.09 ± 0.00 | 0.11 ± 0.01 | 0.35 ± 0.01 | nd | 0.07 ± 0.00 | 0.19 ± 0.01 |
| *Hydrocarbons* | | | | | | | | | | | | |
| Undecane | 0.42 ± 0.00 | 1.76 ± 0.09 | 5.81 ± 0.02 | nd | 0.32 ± 0.01 | nd | 0.04 ± 0.00 | 0.11 ± 0.01 | 0.59 ± 0.02 | 0.41 ± 0.01 | nd | nd |
| Dodecane | 0.88 ± 0.03 | 0.72 ± 0.02 | nd | 0.79 ± 0.02 | 0.66 ± 0.01 | nd | 0.15 ± 0.00 | 0.12 ± 0.01 | nd | 0.45 ± 0.02 | 0.47 ± 0.00 | nd |
| Tetradecane | 0.92 ± 0.01 | 0.82 ± 0.01 | nd | 1.05 ± 0.02 | 0.77 ± 0.02 | 5.61 ± 0.23 | 0.10 ± 0.01 | 0.13 ± 0.01 | nd | 0.39 ± 0.01 | 0.45 ± 0.01 | nd |
| Hexadecane | 1.35 ± 0.08 | 0.94 ± 0.02 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Heptadecane | 1.66 ± 0.07 | 1.07 ± 0.06 | 2.76 ± 0.04 | 1.15 ± 0.04 | nd | nd | nd | nd | nd | nd | nd | nd |
| *Furans* | | | | | | | | | | | | |
| 2-n-Butyl furan | nd | nd | nd | nd | nd | nd | 0.51 ± 0.01 | 0.54 ± 0.01 | 3.34 ± 0.13 | nd | nd | 5.72 ± 0.30 |
| 2-(2-Propenyl) furan | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.45 ± 0.02 | 0.38 ± 0.01 | nd |
| 2-Pentylfuran | 58.99 ± 1.12 | 49.19 ± 0.94 | 394.42 ± 17.32 | 5.27 ± 0.20 | 6.55 ± 0.22 | 111.81 ± 4.07 | 33.88 ± 1.26 | 26.14 ± 1.44 | 237.95 ± 14.74 | 3.85 ± 0.13 | 2.97 ± 0.31 | 104.28 ± 6.29 |
| (*E*)-2-(2-Pentenyl)furan | nd | nd | 5.44 ± 0.21 | 25.74 ± 2.15 | 24.32 ± 1.22 | 110.76 ± 8.52 | nd | 0.15 ± 0.01 | 0.95 ± 0.08 | 38.43 ± 1.34 | 32.18 ± 1.50 | 99.45 ± 0.30 |
| (*E*)-2-(1-Pentenyl)furan | 0.27 ± 0.01 | 0.57 ± 0.02 | 3.23 ± 0.19 | nd | nd | 0.66 ± 0.01 | 0.46 ± 0.00 | 0.37 ± 0.01 | 1.78 ± 0.17 | 0.10 ± 0.00 | 0.06 ± 0.00 | 0.84 ± 0.03 |
| *Esters* | | | | | | | | | | | | |
| Hexanoic acid, ethyl ester | 1.97 ± 0.04 | 1.57 ± 0.13 | 3.68 ± 0.03 | nd | nd | nd | 1.75 ± 0.05 | 1.86 ± 0.02 | 3.95 ± 0.16 | nd | nd | 1.07 ± 0.04 |
| Octanoic acid, ethyl ester | nd | nd | nd | nd | nd | nd | 0.18 ± 0.02 | 0.23 ± 0.01 | 0.45 ± 0.02 | 0.50 ± 0.01 | 0.24 ± 0.02 | 0.91 ± 0.07 |
| Nonanoic acid, ethyl ester | nd | nd | nd | nd | nd | nd | 0.09 ± 0.00 | 0.18 ± 0.01 | 0.31 ± 0.02 | nd | nd | nd |

(*continued on next page*)

**Table 1** (*continued*)

| Volatile compounds [b] | 13-HPOD | | | 13-HPOT | | | 9-HPOD | | | 9-HPOT | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Oat | Barley | Soy bean | Oat | Barley | Soy bean | Oat | Barley | Soy bean | Oat | Barley | Soy bean |
| Butanoic acid, octyl ester | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.31 ± 0.01 | 0.19 ± 0.02 | 1.71 ± 0.01 |
| *Acids* | | | | | | | | | | | | |
| Acetic acid | nd | nd | nd | 0.67 ± 0.02 | 0.14 ± 0.01 | 0.65 ± 0.03 | nd | nd | nd | 0.06 ± 0.00 | 0.05 ± 0.00 | 0.17 ± 0.02 |
| Hexanoic acid | 0.95 ± 0.05 | 1.00 ± 0.02 | 3.52 ± 0.02 | nd | 0.22 ± 0.01 | 2.14 ± 0.07 | 0.68 ± 0.01 | 0.59 ± 0.01 | 1.67 ± 0.18 | nd | nd | 0.86 ± 0.03 |
| Nonanoic acid | 0.61 ± 0.01 | 0.66 ± 0.01 | 2.77 ± 0.18 | 0.95 ± 0.01 | 0.95 ± 0.02 | 4.45 ± 0.09 | nd | 0.02 ± 0.00 | 0.71 ± 0.03 | nd | nd | nd |
| **Total compounds** | 109.36 ± 1.06 | 104.78 ± 0.46 | 978.26 ± 20.53 | 61.38 ± 2.22 | 45.45 ± 1.12 | 649.91 ± 5.93 | 45.25 ± 1.28 | 38.13 ± 1.30 | 322.97 ± 15.66 | 61.06 ± 1.89 | 45.57 ± 2.00 | 301.58 ± 4.38 |

[a] The hydroperoxide substrates included 13-hydroperoxy octadecadienoic acid (13-HPOD), 13-hydroperoxy octadecatrienoic acid (13-HPOT), 9-hydroperoxy octadecadienoic acid (9-HPOD) and 9-hydroperoxy octadecatrienoic acid (9-HPOT); Volatile compounds were calculated as peak area;
[b] nd = not detected.

substrate specificity of HPL in cereals and legumes. Koeduka et al. (2003) used a clone method to study the substrate specificity of fatty acid HPL in barley. They found that HPL activity using the 13-HPOT substrate was over 14 times higher than when using 13-HPOD, but no activity was detected when using either 9-HPOD or 9-HPOT as substrates. Feng et al. (2022) also reported the presence of HPL in soy bean, with an activity of 25.4 μmol $min^{-1}$ $g^{-1}$ using 13-HPOD substrate, which was similar to the results of our study. Mu, Xue, Jiang, and Hua (2012) reported that the optimal substrate for potato, pea seeds and barley was 13-HPOT, while the optimal substrate for *A. thaliana* and cucumber was 13-HPOD. Furthermore, it was inferred that the behavior of HPL highly depends on the hydroperoxide isomerism and unsaturation (Toporkova et al., 2020).

### 3.4. Volatile lipid oxidation compounds induced by HPL in oat, barley and soy bean

Oat, barley and soy bean samples were selected to study the volatile lipid oxidation products induced by HPL. Using 13-HPOD/T and 9-HPOD/T substrates, a total of 37 volatile compounds were identified, including aldehydes, ketones, alcohols, hydrocarbons, furans, esters and acids (Table 1 and Supplementary Table S1). A comparison of the total content of volatile compounds in the samples showed that the volatile production in soy bean (301.58 ± 4.38–978.26 ± 20.53 counts * s * $10^6$) was much higher than that of the oat (45.25 ± 1.28–109.36 ± 1.06 counts * s * $10^6$) and barley (38.13 ± 1.30–104.78 ± 0.46 counts * s * $10^6$) samples. Orthogonal partial least squares discriminant analysis (orthoPLS-DA) was used to verify the results and showed that the differences between soy bean, oat and barley were obvious, as shown in Fig. 3A-a. However, volatile production varied greatly with the different substrates. Clearly, all the samples had greater selectivity toward 13-HPOD/T, whose total amount of volatiles was two to three times greater than that of 9-HPOD/T (Table 1). This may indicate the presence of both 13-HPL and 9-HPL in oat, barley and soy bean and that the 13-HPL-mediated scission of 13-HPOD/T plays a dominant role in the formation of volatile compounds.

When comparing the hydroperoxide substrate selectivity derived from linoleic acid and α-linolenic acid, soy bean HPL showed a preference for linoleic acid hydroperoxides (i.e. 13-HPOD and 9-HPOD). On the other hand, both oat and barley preferred 13-HPOD, although the amount of volatiles produced by 9-HPOT was slightly higher than that produced by 9-HPOD.

Aldehydes and furans accounted for the highest proportions of total volatile compounds, ranging from 15.24%–59.71% and 41.21%–78.11%, respectively, with the main compounds being hexanal and 2-pentylfuran. Studies have shown that aldehydes in oat and soy bean play an important role in the specific flavour even at low amounts (McGorrin, 2019; Xiao et al., 2020). The most probable degradation of linoleic acid and α-linolenic acid under the action of LOX- and HPL-mediated pathway was proposed as shown in Fig. 4, based on our results and the studies of Grechkin (1998), Matsui et al. (2001) and Qian et al. (2016). A large amount of hexanal was observed in oat, barley and soy bean HPL-catalysed degradation of 13-HPOD (Table 1 and Fig. 4A). Hexanal is a typical compound derived from the scission of 13-HPOD that has been found in oat and that indicates lipid oxidation (Yang, Piironen, & Lampi, 2019). Hexanal can also be formed by the 2-alkenal reductase catalysed transfer of (*E*)-2-hexenal (Fig. 4B), which might explain the formation of hexanal by scission of 13-HPOT, especially in soy bean (Table 1). It should be pointed out that of the aldehydes, (*E*)-2-hexenal was another compound with high values resulting from the scission of 13-HPOT in soy bean, but its content in the oat and barley samples was relatively small.

The compound 2-pentylfuran possesses a beany flavour and has been measured at high levels in both cereals and legumes, including oat, barley and faba bean (Lampi et al., 2020; Tang et al., 2023). It has been suggested that this compound is formed as a reaction product of nonenyl radicals derived from the scission of 9-HPOD, as well as a product of 2,4-decadienal (Lampi et al., 2020). Other compounds, such as hydrocarbons, alcohols and ketones, were found in our study (Table 1), and these contributed to the formation of flavours in cereals (McGorrin, 2019). However, their formation in relation to the HPL-catalysed pathway needs further verification.

The variable importance in projection (VIP) method in partial least squares discriminant analysis (PLS-DA) was used to show the role of the 10 most important compounds in classifying and distinguishing the different samples (Fig. 3A-b). A heat map showed the differences between the compound in all the samples (Fig. 3A-c). The heat map showed great differences between soybean-13-HPOD, soybean-13-HPOT and the other groups. In particular, for aldehydes and furans, the red area was more prominent than it was for the other groups, indicating a huge difference in the volatile profiles produced by soy bean, oat and barley.

### 3.5. Formation of volatile compounds by lipid-degrading enzymes in oat, barley and soy bean-based food models

This study evaluated the behavior of lipid-degrading enzymes in the formation of volatile compounds using food models with different pH conditions (pH 5, 6.5 and 8). At pH 5 and 8, the HPL was expected to show low activity (Fig. 2), while at a pH of 6.5, we predicted that the activities would be high for HPL and LOX. RO and ROFA food model systems at 3% were applied based on the procedure established by Lampi et al. (2020), who investigated the function of lipase and LOX in the formation of compounds in faba bean. Thus, for RO, it was expected that lipid degradation would start with hydrolysis by lipase, followed by lipid oxidative enzymes, while for ROFA, no lipase activity would be

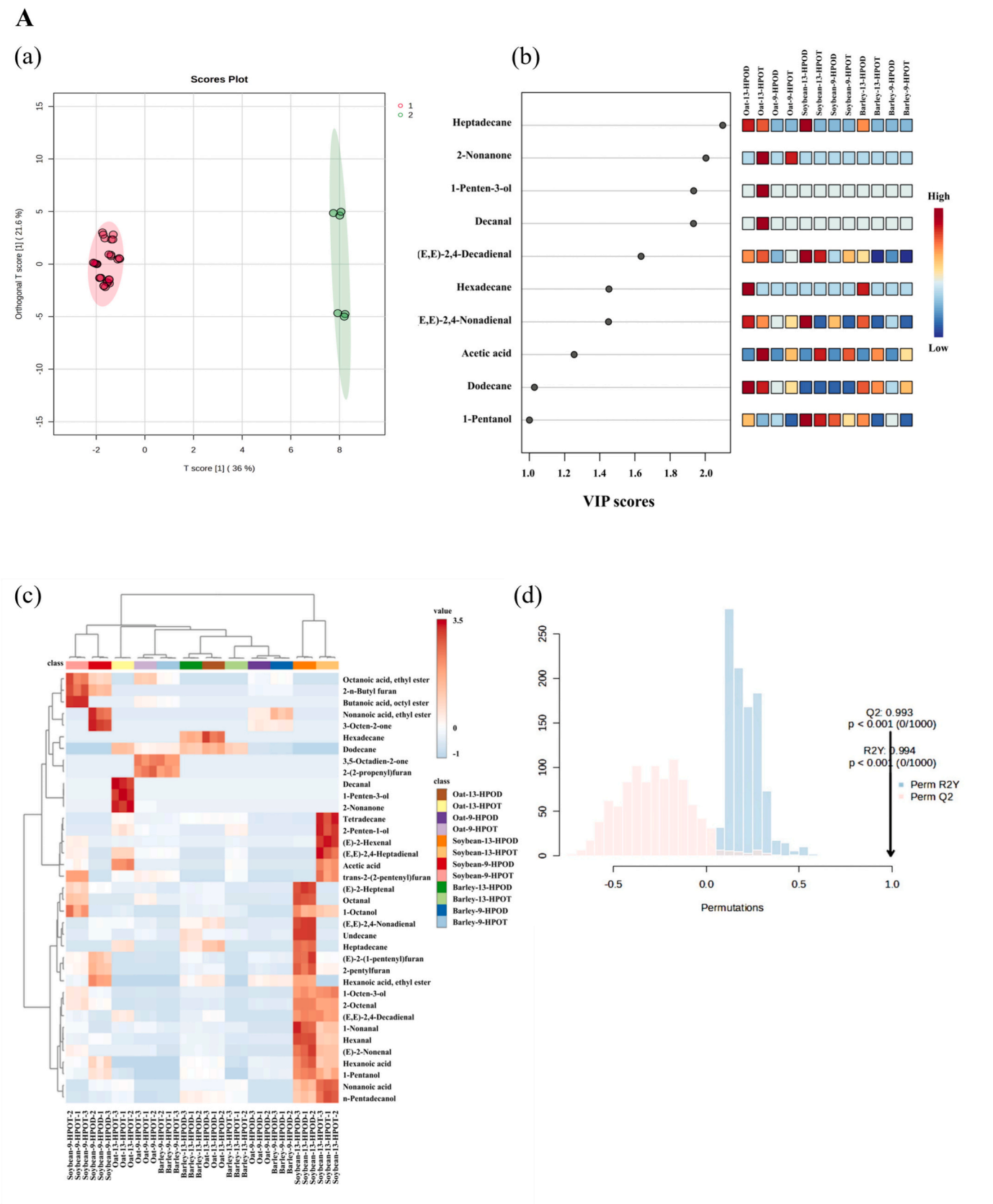


**Fig. 3.** Multivariate analysis of the volatile profiles of: (A) lipoxygenase (LOX)-hydroperoxide lyase (HPL)-mediated volatile compounds, where in figure (a) the number 1 represents oat and barley 9-HPOD/T and 13-HPOD/T, and soy bean 9-HPOD/T, while the number 2 in green represents soy bean 13-HPOD/T; and (B) the volatile compounds in oat, barley and soy bean food models, where in figure (a) the number 2 represents soybean-RO-pH 6.5, soybean-ROFA-pH 6.5 and barley-ROFA-pH 6.5, and the number 1 represents the other samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

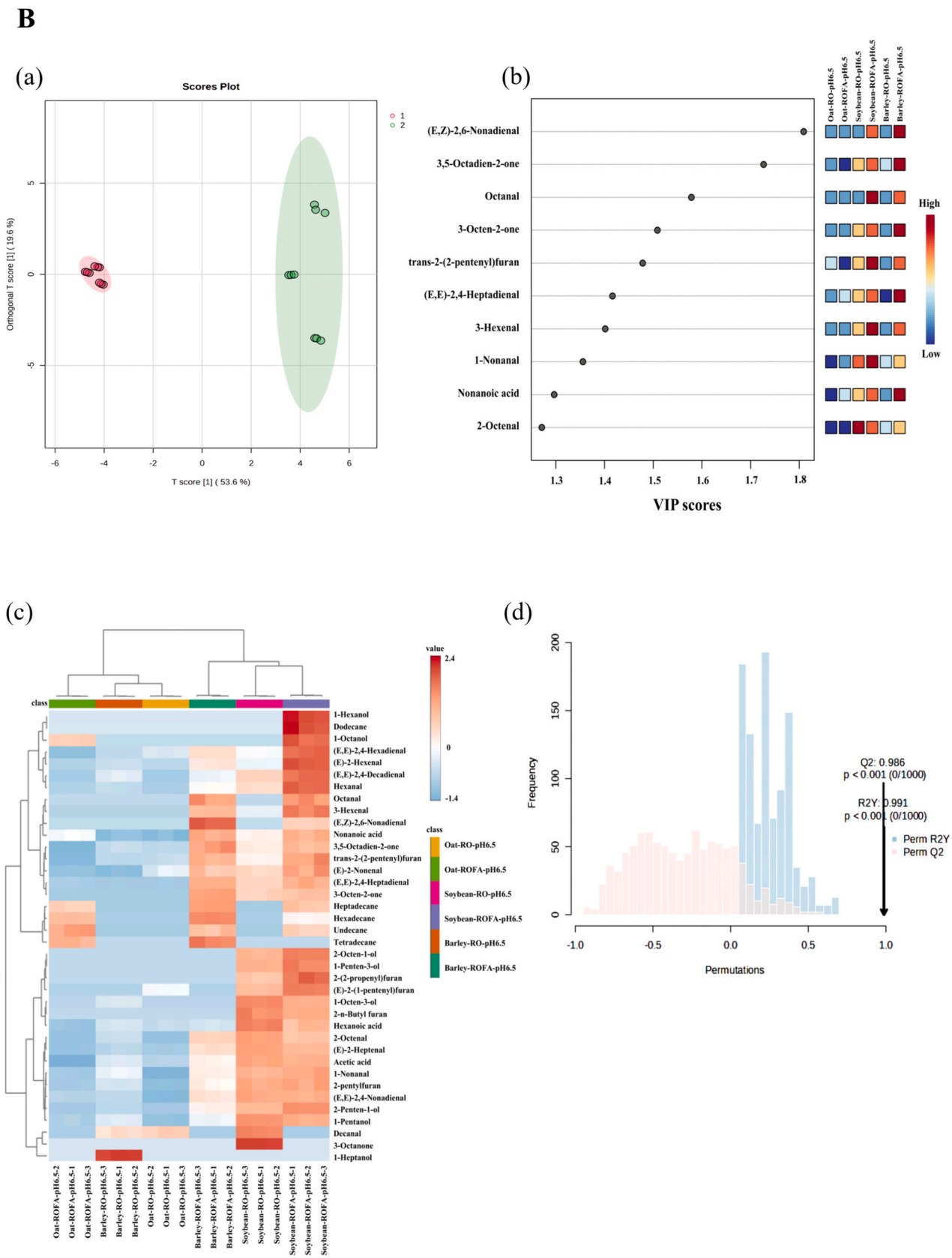


**Fig. 3.** (*continued*).

needed.

As seen in Tables 2a and 2b, the majority of the volatile compounds resulting from the enzyme-catalysed lipid degradation of RO and ROFA in all samples were derived from the oxidation of linoleic acid and α-linolenic acid, as shown in Table 1. In both the RO and ROFA food models, the greatest amount of volatile compounds was produced by soy

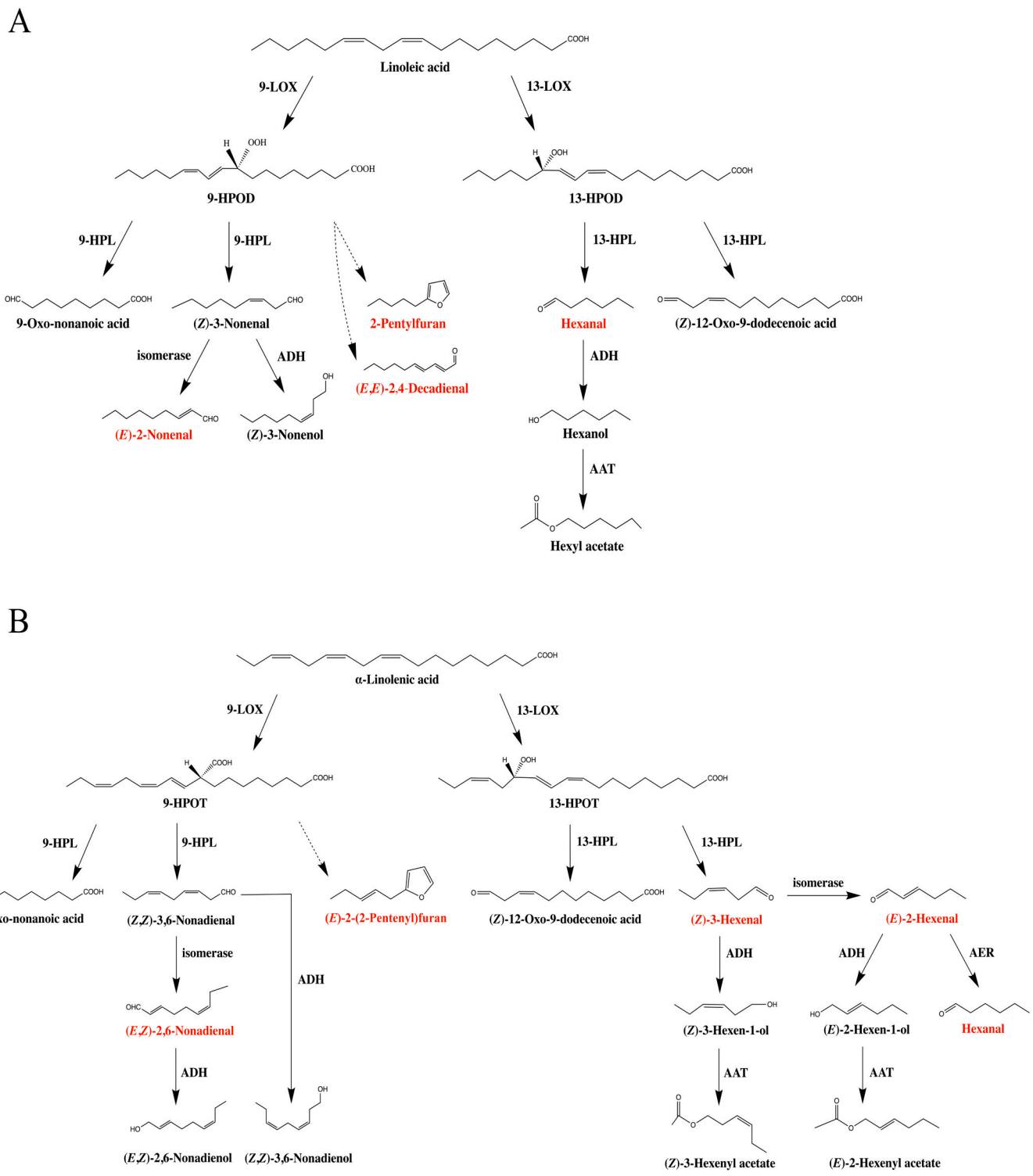


**Fig. 4.** The proposed oxidation mechanism of: (A) linoleic acid and (B) α-linolenic acid under the action of the LOX-HPL-mediated pathways. LOX = lipoxygenase; HPL = hydroperoxide lyase; ADH = alcohol dehydrogenase; AAT = alcohol acyl transferases; AER = 2-alkenal reductase. Red colour indicates the volatile compounds detected in this study. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bean, followed by barley and oat, showing the high potential of lipid-degrading enzymes in soy bean-based foods. Similar to the volatile compounds produced by LOX-HPL-mediated degradation (Table 1), aldehydes and furans were the most abundant when RO was used as the substrate food model, accounting for 34.14%–59.82% and 19.69%–28.73% (Table 2a), respectively. When ROFA was used as the substrate, the values were 36.76%–81.21% and 9.59%–30.29%, respectively (Table 2b). Lampi et al. (2020) also found that aldehydes and furans were the main products in faba bean-based foods, while hexanal and 2-pentylfuran were the main compounds among the lipid oxidation products. Interestingly, a high content of (*E*)-2-hexenal was found in the soy bean and barley ROFA food models, but the (*E*)-2-hexenal content was low in oat-based food models (Table 2b). This may be due to the high 13-HPL activity in soy bean and barley food models.

**Table 2a**
Formation of the identified volatile lipid oxidation compounds in oat, barley and soy bean by refined rapeseed oil (RO) at different pH conditions (counts * s * $10^6$) [a].

| Volatile compounds [b] | RO-pH 5 | | | RO-pH 6.5 | | | RO-pH 8 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Oat | Barley | Soy bean | Oat | Barley | Soy bean | Oat | Barley | Soy bean |
| *Aldehydes* | | | | | | | | | |
| Hexanal | 2.65 ± 0.08 | 13.03 ± 0.52 | 45.64 ± 0.41 | 6.92 ± 0.08 | 14.72 ± 0.38 | 75.40 ± 1.05 | 3.38 ± 0.05 | 13.68 ± 0.21 | 55.89 ± 0.52 |
| (*Z*)-3-Hexenal | nd | nd | 0.68 ± 0.03 | nd | nd | 0.63 ± 0.01 | nd | nd | nd |
| (*E*)-2-Hexenal | nd | 0.98 ± 0.01 | 2.28 ± 0.02 | 1.10 ± 0.04 | 2.29 ± 0.02 | 8.49 ± 0.07 | nd | 2.60 ± 0.06 | 3.19 ± 0.07 |
| Octanal | nd | nd | nd | nd | nd | nd | nd | 0.92 ± 0.01 | 1.18 ± 0.05 |
| (*E*)-2-Heptenal | 0.16 ± 0.01 | 1.19 ± 0.07 | 7.90 ± 0.21 | 0.73 ± 0.01 | 1.15 ± 0.06 | 10.47 ± 0.11 | 0.29 ± 0.01 | 1.17 ± 0.01 | 4.31 ± 0.01 |
| 1-Nonanal | 0.64 ± 0.02 | 2.52 ± 0.01 | 4.06 ± 0.10 | 0.89 ± 0.01 | 2.87 ± 0.09 | 6.38 ± 0.12 | 0.80 ± 0.01 | 2.62 ± 0.05 | 1.36 ± 0.13 |
| (*E,E*)-2,4-Hexadienal | 0.52 ± 0.00 | nd | 1.83 ± 0.06 | 0.53 ± 0.01 | 0.32 ± 0.01 | 0.92 ± 0.01 | 0.08 ± 0.00 | nd | 1.73 ± 0.09 |
| (*Z*)-2-Octenal | nd | 2.63 ± 0.06 | 13.62 ± 0.58 | nd | 2.11 ± 0.06 | 19.73 ± 0.28 | nd | 2.25 ± 0.03 | 12.49 ± 0.15 |
| (*E,E*)-2,4-Heptadienal | 0.17 ± 0.00 | 1.10 ± 0.03 | 2.13 ± 0.06 | 1.09 ± 0.03 | 1.09 ± 0.03 | 7.00 ± 0.06 | 0.19 ± 0.01 | 1.02 ± 0.02 | 2.91 ± 0.02 |
| Decanal | 0.18 ± 0.01 | nd | 0.87 ± 0.01 | 0.20 ± 0.01 | 0.16 ± 0.01 | 0.36 ± 0.01 | nd | 0.16 ± 0.00 | nd |
| (*E*)-2-Nonenal | 0.81 ± 0.03 | 0.34 ± 0.01 | 2.34 ± 0.03 | 1.33 ± 0.08 | 0.63 ± 0.01 | 2.19 ± 0.05 | 0.69 ± 0.01 | 0.52 ± 0.01 | 2.52 ± 0.08 |
| (*E,Z*)-2,6-Nonadienal | nd | nd | 0.22 ± 0.01 | nd | nd | nd | nd | nd | nd |
| (*E,E*)-2,4-Nonadienal | 0.09 ± 0.00 | 0.16 ± 0.00 | 2.20 ± 0.08 | 0.12 ± 0.01 | 0.43 ± 0.01 | 2.62 ± 0.08 | 0.09 ± 0.01 | 0.34 ± 0.02 | 1.79 ± 0.10 |
| (*E,E*)-2,4-Decadienal | nd | 1.32 ± 0.01 | 1.54 ± 0.10 | nd | 1.93 ± 0.08 | 7.24 ± 0.08 | nd | 0.78 ± 0.01 | 3.07 ± 0.08 |
| *Ketones* | | | | | | | | | |
| 3-Octanone | nd | nd | 1.06 ± 0.03 | nd | nd | 0.63 ± 0.00 | nd | nd | 0.57 ± 0.02 |
| 3-Octen-2-one | nd | nd | 2.39 ± 0.02 | nd | nd | 0.47 ± 0.02 | nd | nd | nd |
| 3,5-Octadien-2-one | 0.22 ± 0.00 | 0.21 ± 0.01 | 1.30 ± 0.07 | 0.17 ± 0.00 | 0.20 ± 0.01 | 0.60 ± 0.01 | nd | 0.16 ± 0.01 | nd |
| *Alcohols* | | | | | | | | | |
| 1-Penten-3-ol | nd | nd | 2.87 ± 0.06 | nd | nd | 3.75 ± 0.02 | nd | nd | 1.60 ± 0.01 |
| 1-Pentanol | 0.06 ± 0.00 | 0.52 ± 0.01 | 2.49 ± 0.14 | 0.07 ± 0.00 | 0.55 ± 0.01 | 3.22 ± 0.03 | 0.06 ± 0.00 | 0.41 ± 0.01 | 1.70 ± 0.04 |
| 2-Penten-1-ol | nd | 0.21 ± 0.01 | 2.29 ± 0.10 | nd | 0.16 ± 0.00 | 3.58 ± 0.01 | nd | 0.21 ± 0.01 | 0.76 ± 0.01 |
| 1-Hexanol | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 1-Octen-3-ol | nd | 1.14 ± 0.06 | 10.02 ± 0.40 | nd | 0.73 ± 0.01 | 12.49 ± 0.12 | nd | 0.64 ± 0.01 | 5.38 ± 0.13 |
| 1-Heptanol | nd | 0.50 ± 0.02 | 4.12 ± 0.04 | nd | 0.50 ± 0.00 | nd | nd | 0.51 ± 0.02 | nd |
| 1-Octanol | 0.39 ± 0.01 | nd | 5.68 ± 0.24 | nd | nd | nd | 0.35 ± 0.01 | nd | nd |
| 2-Octen-1-ol | nd | nd | 2.31 ± 0.17 | nd | nd | 0.36 ± 0.01 | nd | nd | 0.36 ± 0.01 |
| *Hydrocarbons* | | | | | | | | | |
| Undecane | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Dodecane | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Tetradecane | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Hexadecane | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Heptadecane | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| *Furans* | | | | | | | | | |
| 2-n-Butyl furan | nd | nd | 3.34 ± 0.04 | nd | nd | 1.06 ± 0.05 | nd | nd | 1.08 ± 0.07 |
| 2-(2-Propenyl)furan | nd | nd | 2.64 ± 0.04 | nd | nd | 0.74 ± 0.02 | nd | nd | 0.49 ± 0.02 |
| 2-Pentylfuran | 1.58 ± 0.02 | 7.35 ± 0.01 | 35.21 ± 1.41 | 2.01 ± 0.03 | 8.24 ± 0.10 | 38.08 ± 1.11 | 2.79 ± 0.11 | 6.63 ± 0.36 | 34.33 ± 0.88 |
| (*E*)-2-(2-Pentenyl)furan | 2.81 ± 0.06 | 1.72 ± 0.08 | 9.69 ± 0.13 | 2.96 ± 0.11 | 2.36 ± 0.07 | 8.49 ± 0.09 | 0.11 ± 0.01 | 2.87 ± 0.16 | 5.88 ± 0.01 |
| (*E*)-2-(1-Pentenyl)furan | nd | nd | 1.42 ± 0.01 | 0.09 ± 0.00 | nd | 0.31 ± 0.01 | nd | nd | 0.65 ± 0.02 |
| *Ester* | | | | | | | | | |
| Hexanoic acid, ethyl ester | nd | nd | nd | nd | nd | nd | nd | nd | 5.51 ± 0.11 |
| *Acid* | | | | | | | | | |
| Acetic acid | 0.69 ± 0.02 | 1.00 ± 0.03 | 5.43 ± 0.10 | 0.59 ± 0.01 | 1.06 ± 0.03 | 4.54 ± 0.08 | 1.06 ± 0.01 | 1.07 ± 0.05 | 3.42 ± 0.21 |
| Hexanoic acid | 3.78 ± 0.09 | 5.06 ± 0.03 | 28.64 ± 1.11 | 4.40 ± 0.07 | 5.05 ± 0.06 | 16.10 ± 0.15 | 4.12 ± 0.07 | 4.82 ± 0.11 | 11.25 ± 0.79 |
| Nonanoic acid | 0.52 ± 0.01 | 0.22 ± 0.00 | 0.33 ± 0.02 | 0.27 ± 0.01 | 0.30 ± 0.01 | 0.59 ± 0.01 | 0.72 ± 0.02 | 0.27 ± 0.01 | 0.47 ± 0.02 |
| **Total compounds** | 15.27 ± 0.26 | 41.22 ± 0.68 | 206.54 ± 3.05 | 23.48 ± 0.33 | 46.84 ± 0.26 | 236.44 ± 3.09 | 14.71 ± 0.17 | 43.66 ± 0.72 | 163.90 ± 2.14 |

[a] Volatile compounds were calculated as peak area;
[b] nd = not detected.

The pH factor had a great impact on the build-up of volatile lipid oxidation products in oat, barley and soy bean food models, indicating the varied formation of volatile compounds in the preparation of different types of food products. Compared to the volatile formation at pH 5 and pH 8, the amount of volatile compounds was the highest at pH 6.5 in all samples, with values of 23.48 ± 0.33 counts * s * $10^6$, 46.84 ± 0.26 counts * s * $10^6$, and 236.44 ± 3.09 counts * s * $10^6$ for oat-based, barley-based and soy bean-based RO food models, respectively (Table 2a). This may be because the optimum pH of HPL was found to be between 6 and 7 for oat, barley and soy bean, along with high LOX activity in this pH range. It was found that the oat samples had the lowest formation of volatile compounds compared to the barley and soy bean samples, which indicates the importance of LOX activity in food models, as the oat food models showed the lowest LOX activity. In addition, in our study, the formation of volatile compounds in soy bean-based food models was high in all three pH conditions (Table 2a and Table 2b), while one previous study reported quite low lipid oxidation products in faba bean foods at pH 8 (Lampi et al., 2020). This may indicate that the role of LOX and HPL isoenzymes varies greatly in different legume-based foods, such as soy bean and faba bean. Soy bean LOX has been shown to be capable of producing 13-hydroperoxide at pH 8.5–9.0 (Schaich et al., 2013), as well as to possess potential for HPL activity in alkali conditions (Fig. 2); however, little is known about the role of HPL in faba bean. Thus, the role of pH and isoenzyme differences should be considered when producing both cereal-based and legume-based foods.

A comparison of the RO and ROFA food models showed that the total amount of volatile compounds formed by the ROFA food models was higher than that formed by the RO food models, although their formation in the oat food models were moderate (17.54 ± 0.10–26.86 ± 0.24 counts * s * $10^6$) (Tables 2a and Table 2b). This may be related to the low LOX activity in oat, resulting in a low production of hydroperoxides

**Table 2b**
Formation of the identified volatile lipid oxidation compounds in oat, barley and soy bean by rapeseed oil fatty acids (ROFA) at different pH conditions (counts * s * $10^6$) [a].

| Volatile compounds [b] | ROFA-pH 5 | | | ROFA-pH 6.5 | | | ROFA-pH 8 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Oat | Barley | Soy bean | Oat | Barley | Soy bean | Oat | Barley | Soy bean |
| *Aldehydes* | | | | | | | | | |
| Hexanal | 4.46 ± 0.12 | 27.88 ± 0.40 | 158.87 ± 0.87 | 8.38 ± 0.10 | 47.30 ± 0.87 | 222.96 ± 4.94 | 9.59 ± 0.10 | 27.11 ± 0.25 | 153.90 ± 3.59 |
| (*Z*)-3-Hexenal | nd | 4.65 ± 0.16 | 10.77 ± 0.66 | nd | 3.90 ± 0.10 | 6.69 ± 0.33 | nd | nd | 1.40 ± 0.10 |
| (*E*)-2-Hexenal | nd | 16.15 ± 0.35 | 93.26 ± 1.16 | 0.41 ± 0.01 | 25.02 ± 1.37 | 123.11 ± 3.80 | nd | 15.09 ± 0.45 | 69.46 ± 1.53 |
| Octanal | nd | 1.54 ± 0.01 | 2.33 ± 0.15 | nd | 1.86 ± 0.12 | 1.92 ± 0.09 | nd | 1.04 ± 0.02 | 1.56 ± 0.04 |
| (*E*)-2-Heptenal | 0.35 ± 0.01 | 3.87 ± 0.07 | 9.03 ± 0.10 | 0.51 ± 0.01 | 5.44 ± 0.13 | 8.67 ± 0.01 | 0.34 ± 0.01 | 1.83 ± 0.05 | 8.48 ± 0.21 |
| 1-Nonanal | 1.63 ± 0.05 | 3.18 ± 0.02 | 5.86 ± 0.09 | 1.43 ± 0.02 | 3.91 ± 0.07 | 6.97 ± 0.19 | 1.32 ± 0.01 | 2.29 ± 0.12 | 2.58 ± 0.12 |
| (*E,E*)-2,4-Hexadienal | nd | 1.23 ± 0.03 | 3.86 ± 0.13 | nd | 1.71 ± 0.01 | 4.82 ± 0.12 | nd | 0.98 ± 0.01 | 5.72 ± 0.24 |
| (*Z*)-2-Octenal | nd | 6.98 ± 0.12 | 15.23 ± 0.14 | nd | 11.37 ± 0.12 | 13.80 ± 0.24 | nd | nd | 14.37 ± 0.20 |
| (*E,E*)-2,4-Heptadienal | nd | 8.22 ± 0.15 | 12.45 ± 0.22 | 1.27 ± 0.04 | 10.50 ± 0.02 | 10.44 ± 0.43 | nd | 4.63 ± 0.11 | 9.69 ± 0.09 |
| Decanal | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| (*E*)-2-Nonenal | nd | 1.73 ± 0.02 | 2.66 ± 0.17 | 0.70 ± 0.01 | 2.57 ± 0.14 | 3.19 ± 0.17 | 0.82 ± 0.02 | 1.81 ± 0.08 | 4.57 ± 0.21 |
| (*E,Z*)-2,6-Nonadienal | nd | 0.60 ± 0.02 | 0.46 ± 0.01 | nd | 0.92 ± 0.02 | 0.39 ± 0.01 | nd | 0.76 ± 0.01 | 0.37 ± 0.02 |
| (*E,E*)-2,4-Nonadienal | nd | 0.94 ± 0.02 | 1.98 ± 0.02 | 0.36 ± 0.01 | 1.40 ± 0.06 | 2.49 ± 0.04 | 0.25 ± 0.01 | 0.74 ± 0.01 | 3.48 ± 0.18 |
| (*E,E*)-2,4-Decadienal | nd | 2.28 ± 0.03 | 13.11 ± 0.80 | nd | 2.39 ± 0.16 | 18.00 ± 0.51 | nd | 1.74 ± 0.11 | 22.14 ± 0.63 |
| *Ketones* | | | | | | | | | |
| 3-Octanone | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 3-Octen-2-one | nd | 0.62 ± 0.02 | 0.47 ± 0.02 | nd | 0.73 ± 0.01 | 0.56 ± 0.01 | nd | nd | 0.64 ± 0.01 |
| 3,5-Octadien-2-one | nd | 1.43 ± 0.06 | 0.80 ± 0.00 | nd | 1.38 ± 0.09 | 1.06 ± 0.04 | nd | 0.56 ± 0.02 | 0.56 ± 0.02 |
| *Alcohols* | | | | | | | | | |
| 1-Penten-3-ol | nd | nd | nd | nd | nd | 4.95 ± 0.17 | nd | nd | nd |
| 1-Pentanol | nd | 0.65 ± 0.01 | 3.21 ± 0.15 | 0.19 ± 0.02 | 0.69 ± 0.02 | 2.66 ± 0.07 | nd | 0.45 ± 0.01 | 2.58 ± 0.15 |
| 2-Penten-1-ol | nd | 1.02 ± 0.02 | 5.28 ± 0.05 | nd | 1.42 ± 0.05 | 5.25 ± 0.05 | nd | 0.90 ± 0.02 | 5.54 ± 0.20 |
| 1-Hexanol | nd | nd | 1.91 ± 0.15 | nd | nd | 2.98 ± 0.14 | nd | nd | 12.32 ± 0.26 |
| 1-Octen-3-ol | nd | nd | 8.63 ± 0.15 | nd | nd | 8.96 ± 0.09 | nd | nd | 6.26 ± 0.17 |
| 1-Heptanol | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 1-Octanol | 0.47 ± 0.01 | nd | nd | 0.71 ± 0.01 | nd | 1.60 ± 0.08 | 0.58 ± 0.01 | nd | nd |
| 2-Octen-1-ol | nd | nd | nd | nd | nd | 0.50 ± 0.00 | nd | nd | 0.28 ± 0.01 |
| *Hydrocarbons* | | | | | | | | | |
| Undecane | 2.56 ± 0.06 | 2.77 ± 0.14 | 2.60 ± 0.07 | 2.88 ± 0.15 | 2.26 ± 0.12 | 1.89 ± 0.07 | 2.12 ± 0.03 | 3.17 ± 0.01 | 1.90 ± 0.04 |
| Dodecane | nd | nd | nd | nd | nd | 1.21 ± 0.09 | nd | nd | 1.03 ± 0.05 |
| Tetradecane | 2.03 ± 0.05 | 2.57 ± 0.07 | nd | 1.93 ± 0.08 | 2.66 ± 0.11 | nd | 1.99 ± 0.05 | 2.14 ± 0.06 | nd |
| Hexadecane | 1.02 ± 0.03 | 0.86 ± 0.01 | 0.79 ± 0.02 | 0.66 ± 0.00 | 0.99 ± 0.01 | 0.31 ± 0.01 | 0.73 ± 0.01 | 0.82 ± 0.02 | nd |
| Heptadecane | 0.92 ± 0.01 | 0.38 ± 0.00 | 0.81 ± 0.01 | 0.52 ± 0.01 | 0.82 ± 0.01 | 0.59 ± 0.02 | nd | 0.66 ± 0.02 | nd |
| *Furans* | | | | | | | | | |
| 2-n-Butyl furan | nd | nd | nd | nd | nd | 0.87 ± 0.01 | nd | nd | 2.51 ± 0.11 |
| 2-(2-Propenyl)furan | nd | nd | 2.67 ± 0.10 | nd | nd | 1.45 ± 0.07 | nd | nd | 2.27 ± 0.15 |
| 2-Pentylfuran | 2.35 ± 0.17 | 7.51 ± 0.15 | 25.45 ± 0.62 | 3.45 ± 0.06 | 17.04 ± 1.30 | 39.85 ± 1.93 | 2.61 ± 0.02 | 13.21 ± 0.50 | 60.30 ± 1.25 |
| (*E*)-2-(2-Pentenyl)furan | nd | 6.46 ± 0.17 | 10.64 ± 0.52 | nd | 19.22 ± 0.67 | 22.94 ± 1.89 | nd | 18.49 ± 1.14 | 18.24 ± 0.90 |
| (*E*)-2-(1-Pentenyl)furan | nd | nd | 0.22 ± 0.01 | nd | nd | 0.52 ± 0.01 | nd | nd | 0.84 ± 0.01 |
| *Ester* | | | | | | | | | |
| Hexanoic acid, ethyl ester | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| *Acid* | | | | | | | | | |
| Acetic acid | nd | 1.64 ± 0.05 | 4.00 ± 0.12 | nd | 1.97 ± 0.06 | 4.14 ± 0.03 | 1.19 ± 0.03 | 1.81 ± 0.04 | 4.96 ± 0.17 |
| Hexanoic acid | 1.29 ± 0.10 | 3.75 ± 0.04 | 8.11 ± 0.05 | 2.93 ± 0.08 | 4.91 ± 0.12 | 12.01 ± 0.49 | 2.94 ± 0.09 | 3.71 ± 0.06 | 17.11 ± 0.37 |
| Nonanoic acid | 0.43 ± 0.01 | 0.67 ± 0.01 | 0.74 ± 0.01 | 0.52 ± 0.01 | 0.89 ± 0.02 | 0.87 ± 0.02 | 0.60 ± 0.01 | 0.72 ± 0.02 | 1.06 ± 0.03 |
| **Total compounds** | 17.54 ± 0.10 | 109.61 ± 0.75 | 406.19 ± 3.93 | 26.86 ± 0.24 | 173.27 ± 0.25 | 538.63 ± 8.13 | 25.08 ± 0.25 | 104.67 ± 1.39 | 436.13 ± 3.39 |

[a] Volatile compounds were calculated as peak area;
[b] nd = not detected.

from PUFAs. A great increase in lipid oxidation products was found when using ROFA in barley- and soy bean-based food models, which was closely related to LOX and HPL activity, and also showed that FFAs were better substrates than TAGs of RO. This could be due to the short incubation time for lipase, which meant that the incomplete hydrolysation failed to produce sufficient free fatty acids. Future studies are needed to investigate the role of LOX-HPL-mediated lipid degradation during long-term storage of food products.

OrthoPLS-DA was performed on the optimal pH of the two substrates, and great differences were found between soy bean, oat and barley (Fig. 3B-a). This was consistent with the results shown in Tables 2a and b, that is, that FFAs were more prone to being oxidised than TAGs. Similarly, VIP in PLS-DA was used to analyse the most significant compounds, including six aldehydes, two ketones, one furan and one acid compound (Fig. 3B-b). The results showed that the aldehydes were quite different in these samples, which might be an important cause of flavour differences. The heat map more intuitively showed the differences in content between the compounds (Fig. 3B-c). The soy bean food models showed great differences from the oat and barley samples.

## 4. Conclusions

This study investigated the occurrence of HPL activity in oat, barley and soy bean. As a downstream enzyme of LOX, HPL plays an important role in lipid oxidation and leads to the formation of various volatile and nonvolatile products based on the reaction with four substrates (i.e. 13-HPOD/T and 9-HPOD/T). Both the barley and oat samples showed high HPL activity when using 13-HPOD/T as substrates, which may indicate the importance of 13-HPL in the formation of volatile compounds, with

aldehydes (e.g. hexanal) and furans (e.g. 2-pentylfuran) as the major products. In addition, the LOX-HPL-mediated pathway showed a dramatic role in lipid oxidation and the build-up of flavours during food processing, as it was found that volatile compounds were produced under various pH conditions in the food models. The maximum amount of volatile compounds was observed at pH 6.5, where both LOX and HPL showed the most activity. Furthermore, compared to the RO food models, the ROFA food models were more prone to oxidation, indicating that lipid hydrolysis by lipase should be taken into consideration for stable food production, especially during long-term storage. Overall, our study investigated the mechanism of the LOX-HPL-mediated pathway for the formation of lipid-derived volatile compounds in oat, barley and soy bean, and increases our knowledge of the generation and control of off-flavours in both cereals and legumes.

## CRediT authorship contribution statement

**Yue Tang:** Writing – original draft, Methodology. **Chenguang Zhou:** Writing – review & editing. **Zhiyang Yu:** Writing – review & editing. **Meng Jiang:** Writing – review & editing. **Haiyan Wang:** Writing – review & editing. **Zhen Yang:** Conceptualization, Supervision; Writing – original draft, review and editing, Project administration.

## Declaration of competing interest

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

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101514.

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