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Comparing the effect of several pretreatment steps, selected to steer (bio) chemical reactions, on the volatile profile of leek (*Allium ampeloprasum* var. *porrum*)

Sophie M. Delbaere, Tom Bernaerts, Flore Vancoillie, Carolien Buvé, Marc E. Hendrickx, Tara Grauwet, Ann M. Van Loey *

Laboratory of Food Technology, Department of Microbial and Molecular Systems, KU Leuven, Kasteelpark Arenberg 22, B-3001, Leuven, Belgium

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ABSTRACT

Volatile compounds in foods can witness the occurrence of (bio)chemical reactions, comprising both enzymatic and non-enzymatic reactions, which can be influenced by processing. This study investigated the effect of different pretreatments aimed at either minimizing, realized by a heat treatment, or inducing, realized by a tissue disruptive treatment, enzymatic reactivities, on the volatile profile of leek. The volatile profiles obtained were then linked to possible (bio)chemical reactions that could have occurred during the treatments. The study showed that different pretreatments led to markedly different volatile profiles, characterized by different (abundances of) volatile compounds. Partial and extensive tissue disruption was achieved by pulsed electric fields at low electric field strength and mixing, respectively. After these tissue disruptive treatments, the volatile compounds could mainly be related to the occurrence of several enzyme-substrate interactions, including conversions of alk(en)yl cysteine sulfoxides by alliinase and of unsaturated fatty acids by lipoxygenase and hydroperoxide lyase. Thermally-induced reactions were also observed to impact the resultant volatile profile. Present study revealed that targeted (pre)treatment allows to steer (bio)chemical reactions towards specific volatile compounds in leek products.

1. Introduction

Flavor, comprising both taste and aroma, is a key term in describing the sensory quality of food products (Barrett et al., 2010; Voilley & Etiévant, 2006; J. Zhang, Qiu, et al., 2021). Flavor chemicals can be grouped as sulfurous compounds, aldehydes, alcohols, hydrocarbons, esters, furans etc. (J. Zhang, Qiu, et al., 2021). Sulfurous compounds are known to possibly exhibit health beneficial characteristics but their existence might also be responsible for the pungent, strong and sulfurous notes of vegetables (Ascrizzi & Flamini, 2020; Lee et al., 2009; Nielsen & Poll, 2004; Sun Yoo & Pike, 1998). Presence of such (off-flavor) notes could eventually impact the selection, consumption and possible aversion of the product (Barrett et al., 2010; J. Zhang, Qiu, et al., 2021). Specific flavor features arise within the food product by manifestation of (bio)chemical reactions, including non-enzymatic reactions and enzymatic reactions. Enzymatic reactions ensue when an enzyme is able to interact with its corresponding substrate, both often present in different compartments of the plant cell, separated by cell membranes (Li et al., 2021). Hence, cell or membrane disruption (e.g., by cutting or mixing) is necessary to facilitate enzyme-substrate interaction (Lee et al., 2009; Li et al., 2021; Resemann et al., 2004; Wang et al., 2008). Non-enzymatic reactions encompass for instance non-enzymatic thermal degradation of substrates such as poly unsaturated fatty acids (PUFAs) and nonprotein sulfur-containing amino acids, derived from cysteine (i.e., alk(en)yl-L-cysteine-sulfoxides (ACSOs)) and of enzymatic reaction products (Christensen et al., 2007; Li et al., 2021; Resemann et al., 2004; Wang et al., 2008).

Allium species, comprising leek, garlic, onions, chives, shallots and scallions are known to possess a distinctive flavor profile which is a consequence of the various flavor precursors, flavor components and enzymes present in those matrices (Bernaert et al., 2012; Hsing, 2002; Li et al., 2021; Mota et al., 2010; Wang et al., 2008). Worldwide, leek (*Allium ampeloprasum* var. *porrum*) is used as flavor enhancer in meal

* Corresponding author.
E-mail addresses: sophie.delbaere@kuleuven.be (S.M. Delbaere), tom.bernaerts@kuleuven.be (T. Bernaerts), flore.vancoillie@kuleuven.be (F. Vancoillie), carolien.buve@gmail.com (C. Buvé), marceg.hendrickx@kuleuven.be (M.E. Hendrickx), tara.grauwet@kuleuven.be (T. Grauwet), ann.vanloey@kuleuven.be, tara.grauwet@kuleuven.be (A.M. Van Loey).

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Abbreviations

AAT	alcohol acetyl transferase
ACSOs	alk(en)yl cysteine sulfoxides
ADH	alcohol dehydrogenase
ALL	alliinase
AMDIS	Automated Mass Spectral Deconvolution and Identification System
EI	electron ionization
HPL	hydroperoxide lyase
HS-SPME-GC-MS	headspace-solid phase microextraction-gas chromatography-mass spectrometry
LOX	lipoxygenase
LV	latent variable
MPP	Mass Profiler Professional
NoPT	no pretreatment
PCA	Principal Component Analysis
PEF	pulsed electric field
PLS-DA	Partially Least Square Discriminant Analysis
POD	peroxidase
PUFAs	polyunsaturated fatty acids
RI	retention index
VID	Variable IDentification coefficient

preparations or ready to heat products (Wang et al., 2008). In this context, leek can be used both as a tissue-based system (e.g., cut leek) and as a disrupted system (e.g., mixed puree-like systems, soups), providing specific flavor properties as a result of different (bio)chemical reactions. The most prominent reactions contributing to the flavor of leek can be categorized into: (i) enzymatic conversions; (ii) reactions related to thermal degradation of ACSOs, PUFAs and enzymatic reaction products; (iii) Maillard reactions and the successive side reactions; (iv) reactions that arise by autoxidation; and (v) thermally induced oxidation of PUFAs (Christensen et al., 2007; Dugravot et al., 2005; Hammer & Schieberle, 2013; Li et al., 2021; Nielsen et al., 2003; Resemann et al., 2004; Rössner et al., 2002; Wang et al., 2008; Zamora et al., 2015). As for the enzymatic conversions, most important reaction pathways present in *Allium* species are firstly, the pyridoxal 5′-phosphate dependent hydrolysis of non-volatile ACSOs, by alliinase (ALL) (S-alk(en)yl-L-cysteine sulfoxide lyase) (EC 4.4.1.4) (Dugravot et al., 2005; Lee et al., 2009; Li et al., 2021; Nandakumar et al., 2018; Nielsen et al., 2003; Nielsen & Poll, 2004; Ovesná et al., 2015; Wang et al., 2008). Both ACSOs and ALL are present in different compartments in the cell, which is in the cytoplasm and vacuole, respectively (Li et al., 2021; Nandakumar et al., 2018; Nielsen et al., 2003; Wang et al., 2008). ALL mediates the conversion of ACSOs (e.g., alliin) by an α,β-elimination, thereby producing alk(en)yl sulfenic acid, pyruvic acid and ammonia (Lee et al., 2009; Nielsen et al., 2003). Alk(en)yl sulfenic acids are unstable and further degrade to thiosulfinates which rapidly rearrange into compounds such as aroma-affecting sulfides that further react at room temperature. This can create a broad range of secondary sulfurous components (Li et al., 2021; Nielsen et al., 2003; Wang et al., 2008; Zhao et al., 2014). Secondly, the conversion of polyunsaturated fatty acids (PUFAs) with a *cis-cis*-pentadiene moiety catalyzed by lipoxygenase (LOX) (EC 1.13.11.12) generates hydroperoxides, after which further reactions are catalyzed by hydroperoxide lyase (HPL), alcohol dehydrogenase (ADH) and alcohol acetyl transferase (AAT). This pathway is responsible for the formation of volatile aldehydes, alcohols and esters which might be perceived as off-flavors depending on the concentration (Engelberth & Engelberth, 2020; Kazimírová et al., 2021; Nielsen et al., 2003).

Intrinsic (bio)chemical reactions can be modified by processing, usually comprising pretreatment, preservation, storage and/or regeneration steps which alter the volatile profile of the final food product (B. Zhang, Qiu, et al., 2021). Pretreatment steps typically include thermal treatments and/or (partial) tissue disruptive treatments. A frequently used industrial pretreatment step is thermal blanching, which inactivates quality-deteriorating enzymes in fresh foods resulting in stabilization and quality retention of the food product (Gonçalves et al., 2007; Nielsen et al., 2003; B. Zhang, Qiu, et al., 2021). Tissue-disruptive treatments can include both extensive or partial tissue disruption, which can be induced by cutting or mixing and a pulsed electric field (PEF) treatment at low electric field strength (<4 kV/cm), respectively. Most research on PEF focuses on treatments at higher electrical field strength to inactivate microbial cells (cell size 1–10 μm) and enzymes guaranteeing shelf-stable products (Kanduser & Miklavcic, 2008; Mañas & Vercet, 2006; Moens et al., 2020; Puértolas et al., 2017). However, PEF at electric field strengths below 4 kV/cm can cause cell membrane permeabilization of plant cells (cell size 40–200 μm), which is irreversible when the electrical field strength exceeds the critical field strength of the tissue. At these lower field strengths, PEF has already been demonstrated to improve cutting and drying effectiveness and to enhance the release of valuable compounds (Aguiló-Aguayo et al., 2015; Blahovec et al., 2017; Kanduser & Miklavcic, 2008; Kumari et al., 2018; López-Gámez et al., 2020a). Moreover, the release of intracellular material as a result of PEF may affect biochemical reactions in plant-based food products (Barba et al., 2015; Knorr et al., 2011; Mañas & Vercet, 2006; Mannozzi et al., 2019; Moens et al., 2020; Puértolas et al., 2017). In this way, PEF could be used as a unique technique to affect biochemical reactivities in a tissue-based system (Barba et al., 2015; Kumari et al., 2018; López-Gámez et al., 2020b; Mannozzi et al., 2019; Puértolas et al., 2012, 2017). It is hypothesized that the implementation of a PEF treatment at low electric strength will cause the membrane enclosing substrate and enzyme to be permeabilized and in that way impact the volatile profile, whereas mixing is hypothesized to cause a far-driven cell disruption affecting the presence of volatiles differently. The effect of PEF at low electrical field strength on the volatile profile is scarcely studied and has, to the best of our knowledge, only been investigated for whole onion in the study of Nandakumar et al. (2018) for *Allium* species.

In this context, the question arises whether flavor-imparting (bio) chemical reactions can be deliberately steered by targeted (sequences of) processing steps. Therefore, in current research, it is intended to investigate the effect of different selected pretreatments on the (flavor-imparting) volatile profile of leek and to link these profiles to possible (bio)chemical reactions that could have taken place during treatment. This 'targeted-steering' approach is, to the best of our knowledge, not the key focus in studies that have already been published. The selection of the (sequence of) steps was based on the minimization (by heat to inactivate enzymes) or induction (by tissue disruptive treatments) of enzymatic reactivities. *In concreto*, three processing sequences were investigated: (i) mixing prior to heating, aimed at inducing enzymatic reactions upon extensive tissue disruption; (ii) PEF treatment prior to heating and mixing, aimed at inducing enzymatic reactions upon partial tissue disruption; and (iii) mixing after heating, in which no enzymatic reactions are expected to occur. The volatile profiles were analyzed by an untargeted volatile fingerprinting technique using headspace-solid phase microextraction-gas chromatography coupled with mass spectrometry (HS-SPME-GC-MS). The results of this study could be of relevance in designing processing conditions in order to deliberately steer (bio)chemical reactivities to obtain an intended volatile profile of leek, and, possibly, of other vegetables in which enzymes and/or substrates are compartmentalized.

2. Materials and methods

2.1. Raw material

Raw leek (*Allium ampeloprasum* var. *porrum*) was purchased from an

agriculture producer on the day of harvesting. Batch variability was verified by acquiring two batches of leek from the same variety (cv. Pluston) at the same seasonal period of the year (i.e., in the first and second week of April 2021) and stemmed from the same location with similar field specificities (Ardooie, Belgium). Physical parameters matched a 40/60 (white/green) ratio which was considered based on industrial relevance and convenience. Until processing, the vegetables were stored in a refrigerator at 3 °C for maximally 5 days.

2.2. Implementation of different pretreatments

Pretreatment steps were conducted in the context of regulating (bio) chemical reactivities. Additionally, a non-pretreated sample was taken into account. Each pretreatment was performed several times and samples were pooled for analysis. A visual representation of the different pretreatments and the goal of each step during the pretreatment is given in Fig. 1.

2.2.1. No pretreatment (NoPT)

Leeks were washed with tap water to remove the remaining soil before tapping dry with paper. Leek stems were cut in three and put into low density polyethylene bags. Subsequently, the bags were frozen in liquid nitrogen and stored in a freezer at −40 °C.

Before analysis, leek stems were mixed in a frozen state using a Grindomixer GM200 (Retsch GmbH, Haan, Germany), pooled together and mixed with saturated NaCl solution (3:2 ($w : v$)) which enables inhibition of enzymes (experimentally verified beforehand, data not shown) without triggering heat-induced changes.

2.2.2. Heating followed by mixing (Heat + Mix)

To inactivate the enzymes, washed leek stems were cut in three, packed in low density polyethylene bags and heat-treated at 95 °C for 18 min in a water bath for which conditions were determined with a qualitative peroxidase (POD) (EC 1.11.1.7) test according to Adebooye et al. (2008) with slight modifications ensuring POD negative activity, which is considered one of the most heat-stable enzymes in vegetables (Kebede et al., 2014; McLellan & Robinson, 1981; Pérez-Calderón et al., 2017). After heat treatment, the bags were cooled in an ice bath for at least 10 min. Mixing the heat-treated leek with demineralized water in a closed Thermomix (Vorwerk, Wuppertal, Germany) (3:2 ($w : v$)) at the highest speed (i.e., 10,700 rpm) for 1 min created a disintegrated system necessary for analytical purpose. The above steps were repeated until all leeks were treated. Subsequently, all processed samples were pooled and divided in a cooling room (3 °C) into new 50 mL transparent polyethylene terephthalate tubes with a polyethylene cap. Finally, the tubes were frozen in liquid nitrogen and stored at −40 °C until analysis.

2.2.3. Mixing followed by heating (Mix + Heat)

Washed leek was treated in a closed system using a Thermomix by mixing 400 g of leek with demineralized water (3:2 ($w : v$)) for 1 min at 10,700 rpm. Subsequently, an incubation step for 1 h at room temperature (i.e., 22 °C) was implemented, hypothesized to allow enzymatic conversions and was stopped after 1 h by heating the sample in the closed Thermomix to 95 °C for 11 min until POD negative activity. While heating, the sample was gently stirred. Afterwards, the processed sample was cooled in an ice bath for at least 10 min. These processing steps were repeated until sufficient material was treated. Subsequent pooling, tube filling and freezing until analysis were similar to the conditions described in section 2.2.2.

2.2.4. Pulsed electric field followed by heating and mixing (PEF + Heat + Mix)

PEF treatment was executed in a batch type pulsed electric field unit (Cellcrack III, Elea-DIL, German Institute for Food Technologies, Quackenbrück, Germany). The PEF unit with a capacity of 1.0 μF was equipped with a medium-sized treatment chamber consisting of two parallel stainless steel electrodes (20.0 × 20.5 × 0.5 cm, $w \times h \times t$). The interelectrode distance of the electrodes amounted to 29.7 cm and the volume of the treatment chamber was 12.2 L. PEF parameters were optimized and standardized beforehand. Standardized tap water was used as conductive medium (600 μS at 22 °C) and was made by adding 1.3376 g NaCl and 0.2006 g $CaCl_2 \cdot H_2O$ to 5 L of ultrapure Milli-Q water. Two washed leek stems were cut in half, weighed and were inserted next to each other, perpendicular to the electrodes, into the treatment chamber which was filled with standardized tap water until a total mass of 5 kg was obtained. The leek parts were submitted to 30 monopolar (exponential decayed) pulses with a pulse amplitude of 30 kV (i.e., maximum voltage of the PEF equipment) and an electric field strength of 1.01 kV/cm. The number of pulses was based on preliminary tests, in which the leek was subjected to different number of pulses during a PEF treatment after which the treated leek was inserted in a conductive medium of which the conductivity was measured with a Testo®240 conductivity meter with cell type 07 mS (Testo, Lenzkirch, Germany). The number of pulses was selected after which no significant increase in conductivity was observed after a certain time (data not shown). The energy input per pulse, the specific energy input per pulse and the total specific energy input amounted to 450 J/pulse, 90 J/kg·pulse and 2.7 kJ/kg, respectively. The pulse width and pulse frequency were 225 ± 19 μs and 2 Hz, respectively and were acquired using an online digital oscilloscope (Tektronix, Köln, Germany). After treatment, leek stems were kept at room temperature (i.e., 22 °C) for exactly 1 h to enable possible (enzymatic) reactions. Afterwards, the exact same heat, mixing and subsequent storage steps were followed as described in section 2.2.2.

2.3. Analysis of the volatile profile

The volatile profiles of samples were analyzed by means of an untargeted semi-quantitative headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) fingerprinting approach.

2.3.1. Sample preparation

Samples were thawed overnight in a cooling room at 3 °C. For Mix + Heat, Heat + Mix and PEF + Heat + Mix, 0.8 g of thawed sample was brought into a 10 mL amber glass vial (VWR International, Radnor, PA, USA), to which 3 mL saturated NaCl solution and 0.2 mL demineralized water were added. To allow comparison, for the NoPT system (made with saturated NaCl solution (3:2 ($w : v$)), a similar ratio of leek, saturated NaCl solution and demineralized water was ensured. Therefore, the 10 mL vial was filled with 0.8 g of NoPT sample, 2.68 mL of saturated NaCl solution and 0.52 mL of demineralized water. The maximal

Induction enzymatic reactions	Inactivation of enzymes	Sample preparation	Code of sequence of steps
Mix → by extensive tissue disruption	+ Heat		= Mix + Heat
PEF → by partial tissue disruption	+ Heat	+ Mix	= PEF + Heat + Mix
	Heat	+ Mix	= Heat + Mix

Fig. 1. Visual representation of the applied pretreatments.

amount of sample to be inserted into the vial was determined via preliminary tests using dilution series in order to prevent fiber saturation (data not shown). The vials were tightly closed using metal screw-caps with a PTFE/silicone septum seal (Grace, Columbia, MD, USA). To each vial, 100 μL of diluted 3-heptanone solution was added as internal standard. For each type of pretreatment, six replicates were analyzed which was predetermined based on the stagnation of the standard error (data not shown). Since batch variability was seen to be negligible, data from both batches were combined together resulting in 12 replicates per pretreatment.

2.3.2. HS-SPME-GC-MS fingerprinting

The HS-SPME-GC-MS method was adapted from the method as described by Kebede et al. (2015). Incubation time, extraction time and temperature were optimized beforehand using an experimental design aiming to maximize the number of peaks and the total peak area (data not shown). The prepared vials were homogenized and transferred to the cooling tray of the CombiPal autosampler (CTC Analytics AG, Zwingen, Switzerland) which was maintained at 10 °C. Analyzing the volatile profile was executed with a gas chromatographic system (GC 7890B, Agilent Technologies, Santa Clara, CA, USA) coupled with a mass selective detector (MSD) (5977A, Agilent Technologies, Santa Clara, CA, USA). Incubation under agitation at 500 rpm and extraction were performed at 40 °C for 8 min and 20 min, respectively. The volatiles were extracted using a specific 30/50 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (StableFlex, Supelco, Bellefonte, PA, USA). Prior to extraction, the fiber was preconditioned according to the manufacturer instructions. Desorption of volatiles was done at the GC-injection port at 230 °C for 2 min and injection took place in a split mode (1:5). The volatile compounds were chromatographically separated using a HP Innowax column (Agilent Technologies J&W, Santa Clara, CA, USA) (60 m × 0.25 mm i.d., 250 μm d_f). Helium (purity ≥ 99.9999%) was used as a carrier gas with a constant flow of 1.273 mL/min and a pressure of 138.13 kPa. A specific oven program was programmed with a starting temperature of 40 °C which was maintained for 2 min. This was followed by heating to 120 °C at 4 °C/min, heating to 200 °C at 7 °C/min, holding for 2 min at 200 °C and heating to 250 °C at 50 °C/min. The temperature of the ion source and quadrupole amounted to 230 °C and 150 °C, respectively. Mass spectra could be obtained by electron ionization (EI) mode at 70 eV with a scanning range of 35–400 m/z at 3.9 scans/s. Control samples were added in each sequence in order to monitor possible fiber degradation and performance of the analytical instrument. In each sequence, samples were randomly analyzed.

2.4. Multivariate data analysis

Volatile data were analyzed in a similar way as discussed by Vervoort et al. (2012). Automated Mass Spectral Deconvolution and Identification System (AMDIS) (Version 2.72, 2014, National Institute of Standards and Technology, Gaithersburg, Maryland, USA) and Mass Profiler Professional (MPP) software (version B12.00, 2012, Agilent Technologies, Diegem, Belgium) allowed pre-processing the data by deconvoluting the complex chromatograms to receive pure component spectra and to apply peak filtering and aligning. Also, AMDIS enabled integration of chromatograms and was used to build a retention index (RI) calibration file for which homologous series of n-alkane standards (C8–C20) were made which were subjected to the same GC-MS conditions as described in section 2.3.2. Identity of the detected compounds was determined by comparing mass spectra to reference mass spectra in the spectral library of NIST software (NIST14, version 2.2, National Institute of Standards and Technology, Gaithersburg, MD, USA) (Buvé et al., 2018; Ren et al., 2019). MPP yielded the creation of a 2D data table representing data in peak areas with aligned retention times (Aganovic et al., 2016). Solo software (Version 8.7.1, 2020 Eigenvector Research, Wenatchee, WA, USA) was used for mean-centering and weighing of the variables by their standard deviation to give them equal variance. In addition, a principal component analysis (PCA) was performed to detect potential outliers in the data. Afterwards, in order to assess the impact of different pretreatment steps, Partially Least Square Discriminant Analysis (PLS-DA) was performed, a regression based supervised classification method that aims to maximize covariance between *X* (variables, volatile compounds) and *Y* (differently processed samples (i.e., categorical variables, classes)) in the model calculated. The model with the lowest number of latent variables (LVs), resulting in an optimal class separation elucidated by the percentage of variance explained and the root-mean-square error of cross-validation (RMSECV) was selected (Kebede et al., 2014). The Venetian blinds was used as cross-validation method. In order to assess differences among classes in a qualitative way, biplots were constructed using OriginPro8 (Origin Lab Corporation, Northampton, MA, USA) which combines a score plot and a loading plot. Discriminant volatiles were quantitatively selected based on Variable IDentification coefficients (VIDs) which are quantitative measures indicating the correlation between the original *X*- and *Y*-variable(s) as designed by the model (Grauwet et al., 2014; Kebede et al., 2014; Koutidou et al., 2017). Volatiles with VIDs with absolute values between 0.800 and 1.0 were selected in this study and were referred to as discriminant volatiles (markers). Discriminant compound plots were plotted depicting the mean peak area as a function of treatment. Confirmation of the identity of the markers was performed by comparing the RI with those found in available literature and by verifying the match factor, for which the threshold was set on 80%. If the RI was not found in literature or did not match the value described in literature, corresponding compounds were defined as '*tentatively identified*' and '*unidentified*', respectively. In addition, for a selected set of markers, confirmation was done using analytical standards (i.e., pentanal, dimethyl disulfide).

2.5. Statistical analysis

Statistical analyses were conducted using Tukey's HSD tests in JMP Software (JMP Pro16, SAS Institute Inc., Cary, NC, US) (p-value of 0.05) to perform significance tests between the mean peak areas of the discriminant volatiles depicted in the discriminant compound plots.

3. Results and discussion

3.1. Qualitative and quantitative classification of the volatile components of differently pretreated leek samples

The volatile profiles of the differently pretreated samples consisted of 137 volatiles (including internal standard) over all chromatograms for which data sets of peak areas were obtained by integration. Representative total ion chromatograms of the headspace volatile profiles can be found in the supplementary material. By performing PCA, one outlier was removed from the dataset. In Fig. 2, biplots of the PLS-DA model are shown. Three LVs were selected, explaining 97.73% of the total *Y*-variance. Since each LV explained an equal proportion of the *Y*-variance, all three plots are shown.

On the biplots, a depiction of the groups (samples) is made and this visualization illustrates how different samples are related to each other. The distance among samples is a measure for the difference based on volatile profiles. The closer the groups are positioned to each other, the more similar their volatile characteristics. It can be clearly observed that the three pretreatments have led to distinctively different volatile profiles among each other and in comparison with the headspace of the NoPT sample, since clear separate groups can be distinguished on the biplots. Vectors on the biplot give the correlation loadings pointing to the different groups. The longer the vector, the more the volatile profile of that class the vector is pointing to is explained by the PLS-DA model and the more specific the volatile characteristics of that group.

Volatiles are presented as open circles. The more a volatile is depicted into the direction of a vector, the more this volatile is

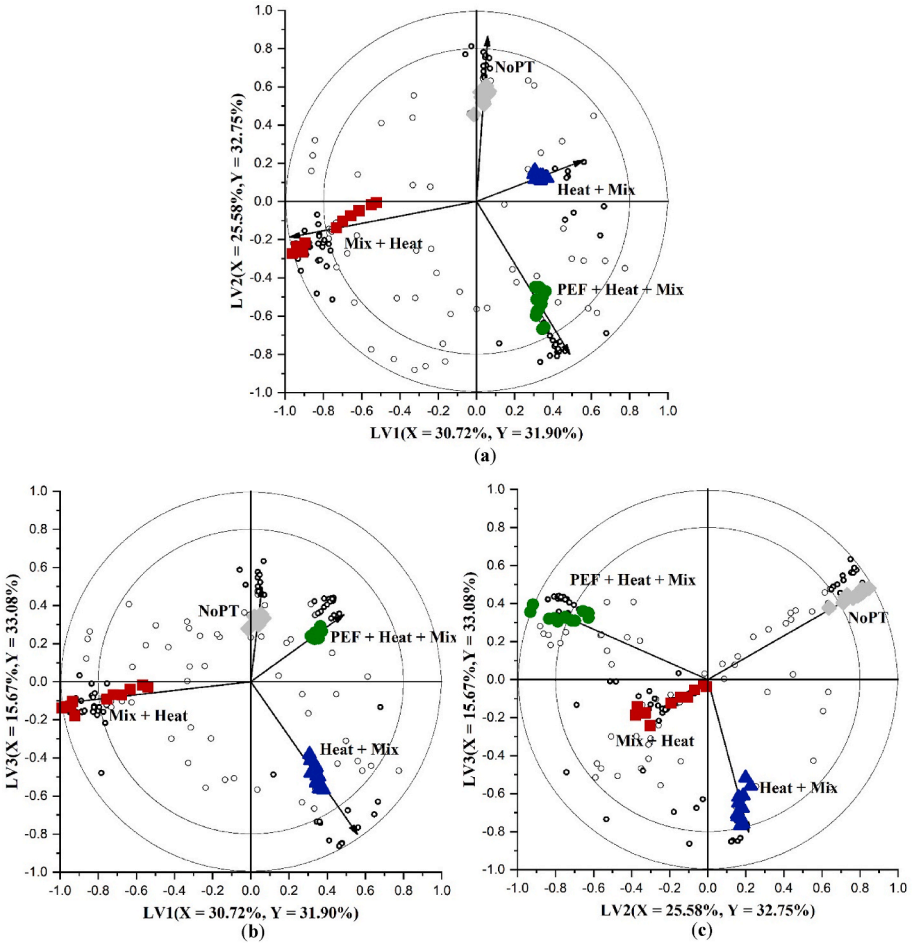

Fig. 2. PLS-DA biplots visualizing the effect of pretreatment (i.e., (◆) No pretreatment (NoPT), (■) Mix + Heat, (●) PEF + Heat + Mix, and (▲) Heat + Mix) on the volatile profile of leek. Open circles (o) on the biplots represent the headspace components for which the components that discriminate between treatments (i.e., discriminant components) are marked in bold ($|VID| \geq 0.800$) (**o**). Vectors depict the correlation loadings for the categorical *Y*-variables. The variance explained by each LV is indicated in the respective axes. The inner and outer circles depict the correlation coefficients of 0.800 and 1.0, respectively. (**a**) LV2 as a function of LV1; (**b**) LV3 as a function of LV1; (**c**) LV3 as a function of LV2.

responsible for describing the variability between the samples in the data set and the more this volatile is representative of the respective group. In contrast, volatiles positioned near to the center are less characteristic for a specific group. As can be observed from Fig. 2, many volatiles are representative for the volatile profile of one specific group illustrating the distinctive profiles. In order to select discriminant volatiles (i.e., markers) out of the large data set, the VID procedure was implemented. This procedure allocates a quantitative coefficient ranging from −1 to 1 to each of the variables in a specific class. Volatiles having a positive VID encompass a higher representation in the specified class while negative values of this coefficient present a lower representation in the respective sample. The VID threshold was set on 0.800 resulting in an amount of 75 discriminant components (Table 1) which comprised 62% of the total peak area of all components detected in the headspace of all samples. A |VID| higher than 0.800 means more than 80% of the variability of a variable located between the 0.800 and 1.0 confidence circle is explained by the LVs used to build the model. The discriminant compounds mentioned in Table 1 are indicated in bold in Fig. 2.

3.2. Interpretation of the identity of selected markers

In the current study, the impact of several pretreatments on the volatile profile of leek was investigated. Since literature regarding the effect of these specified pretreatments, consisting of a specific selected set of steps, is, to the best of our knowledge, nonexistent for leek, it is challenging to unequivocally ascribe a volatile compound to a specific reaction pathway. However, hypothesis-driven links to reaction pathways are being formulated. Immediate heating was expected to prevent enzymatic conversions while both Mix- and PEF-treating the matrix were expected to cause different degrees of tissue disruption enabling volatile affecting substrate-enzyme reactions to a different extent and causing different (abundances of) volatile compounds. It must be noted that prior slicing of the leek (3 slices/stem) was inevitable which could potentially induce biochemical reactions to a minor extent in samples in which induction of enzymatic reactivities was not intended (i.e., Heat + Mix and NoPT) (Li et al., 2021; Nielsen et al., 2003). In addition, the heat applied to inactivate enzymes can also affect the composition of the headspace, which will also be taken into consideration in this study. This again shows the complexity to ascribe a compound to (a) specific reaction pathway(s) due to complex (bio)chemical reactions and the existing inter-reactivity of substrates, intermediates and products which are induced by processing. In the next paragraphs, for each chemical class, interpretation of possible (bio)chemical reactions arisen during pretreatments that led to the selected discriminant compounds will be discussed. As for the selection of the discussed compounds, the focus was laid on markers which could potentially be derived from (bio)chemical conversions related to the ACSOs-ALL pathway on the one hand and the PUFAs-LOX-HPL pathway on the other hand. To ameliorate understanding, specific compound plots which depict how mean peak areas of discriminant volatiles differ between the implemented pretreatments, are included (Fig. 3 and Fig. 4).

Table 1
VID, identity, chemical class and RI of markers (|VID| ≥ 0.800) for differently pretreated leek samples.*

NoPT				
VID	Identity	Chemical class	RI	Odor description
0.969	Acetaldehyde	Aldehyde	632	Fresh, green [b]
0.966	2-Ethyl-trans-2-butenal	Aldehyde	1167	
0.956	2-Methyl-2-pentenal	Aldehyde	1171	Green, grassy, herbal, cabbage, paint, prickling, powerful, slightly fruity odor [c,d,e]
0.955	Hexanal	Aldehyde	1096	Green, grassy, green tomato [b,f]
0.945	4-Methyl-3H-1,2-dithiol-3-one [a]	Ketone	2001	
0.936	*Unidentified*	-	1276	
0.888	(E)-4-Heptenal	Aldehyde	1255	Biscuit, cream [g]
0.881	Propanethial-S-oxide [a]	Aldehyde	1233	Trans: green or raw onion taste and sweet sulfur taste/Cis: sweet or brown 'sauté', hydrogen sulfur notes [c]
0.841	3-Hexenal	Aldehyde	1155	Green leaves, grassy, green, apple-like, leaf-like, cut grass [b,h]
0.834	2-Ethyl-1-hexanol	Alcohol	1498	
0.816	*Unidentified*	-	1011	
0.801	1H-Tetrazol-5-amine [a]	Amine	1977	
−0.807	Dimethyl sulfide	Sulfurous compound	710	Rotten, cooked vegetables, leek, spicy, cabbage, sulfur, gasoline, asparagus-like, putrid [b,g,i]
−0.878	2,4-Dimethyl thiophene	Sulfurous compound	1203	

Mix + Heat				
VID	Identity	Chemical class	RI	Odor description
0.991	(E)-1-Allyl-2-(prop-1-en-1-yl)disulfide	Sulfurous compound	1482	
0.985	(E)-1-Methyl-2-(prop-1-en-1-yl)disulfide [a]	Sulfurous compound	1307	Strong raw onion, leek [c,g]
0.982	(Z)-1-Allyl-2-(prop-1-en-1-yl)disulfide	Sulfurous compound	1500	
0.981	Methyl propyl trisulfide	Sulfurous compound	1549	
0.976	2-Pentylfuran	Furan derivate	1243	Floral, fruit [f]
0.975	(Z)-1-Methyl-2-(prop-1-en-1-yl)disulfide [a]	Sulfurous compound	1281	Strong raw onion, leek [c,g]
0.973	1-Allyl-3-propyl trisulfide	Sulfurous compound	1753	
0.952	2,4-Dimethylfuran	Furan derivate	972	
0.931	(E)-1-(Prop-1-en-1-yl)-3-propyl trisulfide	Sulfurous compound	1766	
0.926	Methyl-2-propenyl trisulfide	Sulfurous compound	1611	
0.924	Dimethyl trisulfide	Sulfurous compound	1404	Solvent, rotten onion, tainted, leek, metal, fish, sulfur, cabbage [c,d,h,i]
0.912	Iodoacetylene [a]	Alkyne	1623	
0.912	*Unidentified*	-	1592	
0.906	1-Allyl-2-isopropyl disulfide [a]	Sulfurous compound	1434	
0.901	*Trans*-β-ionone	Ketone	1976	
0.883	Methyl-2-propenyl disulfide	Sulfurous compound	1299	Fresh garlic [j]
0.882	3,4-Dimethyl thiophene	Sulfurous compound	1270	
0.866	1-((E)-Prop-1-en-1-yl)-2-((Z)-prop-1-en-1-yl)disulfide [a]	Sulfurous compound	1740	
0.862	4-Ethyl benzaldehyde [a]	Aldehyde	1731	Almond, fruity [j]
0.851	Cathinone[a]	N-compound	2019	
0.850	Dimethyl disulfide	Sulfurous compound	1089	Strong, raw onion, sulfuric, fresh leek, cabbage, putrid [c,h,i]
0.848	*Unidentified*	-	1869	
0.839	3-Methyl-1-(methylthio)butane [a]	Alkane	1634	
0.839	Prop-1-enyl dithiopropanonate [a]	Sulfurous compound	1569	
0.833	Propanethioic acid, S-pentyl ester [a]	Ester	1461	
0.833	n-Caproic acid vinyl ester [a]	Ester	133	
0.832	(Z)-1-Methyl-3-(prop-1-en-1-yl)trisulfide [a]	Sulfurous compound	1622	
0.829	2-Methyl pentanoic acid	Carboxylic acid	1813	
0.817	*Unidentified*	-	1140	
0.813	1-Methyl hydrazinecarbodithioic acid, methyl ester [a]	Ester	1748	

PEF + Heat + Mix				
VID	Identity	Chemical class	RI	Odor description
0.991	1-Methylethyl propyl disulfide	Sulfurous compound	1401	
0.991	Allyl-n-propyl sulfide	Sulfurous compound	1121	
0.989	Ethanethioic acid, S-propyl ester	Ester	1190	
0.987	(E)-2-Methyl-2-butenal [a]	Aldehyde	1109	Rancid, cooked vegetables, onion [d]
0.985	(Z)-2-Hexen-1-ol, acetate	Alcohol	1349	
0.982	1-Allyl-2-isopropyl disulfide [a]	Sulfurous compound	1449	
0.980	(Z)-3-Hexen-1-ol, acetate	Alcohol	1332	
0.977	(E)-1-(Prop-1-en-1-yl)-3-propyltrisulfide	Sulfurous compound	1803	
0.977	(E)-2-hexen-1-ol, (E)-	Alcohol	1421	Green, leaf, walnut [i]
0,970	Acetic acid, hexyl ester	Ester	1285	
0.969	(E)-1-(Prop-1-en-1-yl)-3-propyltrisulfide	Sulfurous compound	1824	
0.957	1-Hexanol	alcohol	1367	Resin, flower, green [i]
0.949	1-Methylethyl propyl disulfide	Sulfurous compound	1401	
0.944	*Unidentified*	-	1114	
0.922	2-Ethyl[1,3]dithiane [a]	Sulfurous compound	1540	
0.918	*Unidentified*	-	2055	
0.887	Dichlorofluoromethyl silane [a]	Halogen compound	1618	
0,850	Propanoic acid, 4-hexen-1-yl ester [a]	Ester	1291	

(*continued on next page*)

Table 1 (*continued*)

PEF + Heat + Mix				
VID	Identity	Chemical class	RI	Odor description
0.835	Propyl mercaptan	Sulfurous compound	836	
0.827	1-Propanol	Alcohol	1045	Alcohol, pungent [h,i]
0.804	3-Ethyl-5-methyl-1,2,4-trithiolane [a]	Ester	1715	
Heat + Mix				
VID	Identity	Chemical class	RI	Odor description
0.981	*Cis*-1,3-dimethylcyclohexane	Cycloalkane	815	
0.973	Pentane	Alkane	496	
0.972	Methylcyclohexane	Cycloalkane	747	
0.971	(E)-2-Octene	Alkene	868	
0.934	*Trans*-1,2-dimethylcyclohexane	Cycloalkane	839	
0.930	Pentanal	Aldehyde	985	Grass, banana, aldehyde [h]
0.881	Octane	Alkane	801	
0.871	2,2,4,6,6-Pentamethylheptane	Alkane	956	
0.830	*Cis*-1,3-dimethylcyclohexane	Cycloalkane	848	
0.822	Octahydropentalene [a]	Alkene	964	
0.812	3-Methoxy-1-heptene [a]	Alkene	926	

[*] Components, identified using the spectral library of NIST, that do not match with the RI found in literature, are indicated as '*unidentified*'.

[a] Components, for which the RIs are not found in literature are indicated as '*tentatively identified*'. The compounds are listed in decreasing order of VID. A positive VID of a compound for a class conveys the presence of a higher concentration of that compound in that specific class compared to that compound in (an)other class(es) whereas a negative VID denotes a lower concentration for that compound in that specific class. If found in the literature, the odor description of the marker is added.

[b] Hammer and Schieberle (2013).

[c] Nielsen and Poll (2004).

[d] Van Ruth et al. (1995).

[e] Vincenti et al. (2019).

[f] Dong et al. (2008).

[g] Wei et al. (2021).

[h] Bathgate and Miller (2019).

[i] Flavornet (2004).

[j] Li et al. (2021)).

3.2.1. *Sulfurous compounds*

The specific compound plots of the sulfurous compounds described in this section are shown in Fig. 3. Dimethyl disulfide, dimethyl trisulfide, methyl propyl trisulfide, (E/Z)-1-methyl-2-(prop-1-en-yl)disulfide, methyl-2-propenyl trisulfide, methyl-2-propenyl disulfide and (Z)-1-methyl-3-(prop-1-en-1-yl)trisulfide were seen to be abundantly present in the volatile profile after a Mix + Heat treatment. These compounds can have odor notes as strong, onion, solvent, fish, metal, cabbage, sulfur, garlic and putrid, as reported in literature (Bathgate & Miller, 2019; Flavornet, 2004; Ghita Studsgaard Nielsen & Poll, 2004; Van Ruth et al., 1995; Wei et al., 2021). Observing these compounds, extensive cell disruption clearly allowed ALL to react with its corresponding substrates due to decompartmentalization. This disruption led to the formation of various compounds derived from ACSOs (e.g., thiosulfinates), which rapidly rearranged into a mixture of sulfurous components such as the observed di- and trisulfides (Ascrizzi & Flamini, 2020; Dugravot et al., 2005; Li et al., 2021; Mellouki et al., 1994; Resemann et al., 2004; Sun Yoo & Pike, 1998; Wang et al., 2008). Dimethyl disulfide and dimethyl trisulfide on the one hand and methyl propyl trisulfide on the other hand have already been reported as major volatile compounds imparting the odor of onion and leek, respectively by Wang et al. (2008) and Schulz et al. (1998). Besides the ALL-catalyzed formation, the latter components as well as dimethyl sulfide might have been derived from thermally degraded enzymatic reaction products and/or pathways of thermal degradation of methyl cysteine sulfoxide (Li et al., 2021; Rössner et al., 2002). It has been reported that methanesulfenic acid in which thermally degraded methyl cysteine sulfoxide can be converted, can undergo self-condensation into the unstable thiosulfinate which subsequently decomposes mainly into dimethyl disulfide and dimethyl trisulfide (Rössner et al., 2002).

Thermal pathways were believed to be the main routes that led to the formation of dimethyl sulfide as observed in the significant higher abundance in the headspace after a Heat + Mix pretreatment (Fig. 3). Dimethyl sulfide might also be enzymatically formed after a mix step and a PEF step, but might already be further degraded in the following steps during treatment since no significant higher abundance of this compound was observed in the headspace after treatments that intended to induce enzymatic reactivities. Besides, since the abundance of this compound was higher in both headspaces after Heat + Mix and PEF + Heat + Mix compared to its abundance in the headspace after a Mix + Heat treatment, the physical state on which the heat step was applied might also have been a determinative factor in the effect of thermal degradation of substrates and/or reaction products leading to this compound. More specifically, it seemed that the heat impact on a tissue-based system (i.e., PEF-treated leek and untreated leek) had more effect on the presence of dimethyl sulfide compared to the mixed leek sample. Dimethyl disulfide and dimethyl trisulfide resulted mainly as a result of enzymatic conversion (and/or as a result of heat-induced degradation of enzymatically formed products) after a mix step since these compounds were less observed in the volatile profile after a Heat + Mix treatment. Moreover, to generate these compounds, a more intense tissue disruptive step seemed necessary, given that those compounds were not abundant after a PEF + Heat + Mix pretreatment. The fact that the abundance is significantly higher in the volatile profile of Mix + Heat compared to PEF + Heat + Mix can additionally be explained by the difference in physical state on which the heat step was applied leading to a different degree of possible thermally-induced reactions leading to these compounds.

In addition, (E)-1-allyl-2-(prop-1-en-1-yl)disulfide and 1-allyl-3-propyl trisulfide were also observed to be abundantly present in the headspace after a Mix + Heat treatment (Fig. 3). As implicated by Li et al. (2021), allyl sulfides (e.g., diallyl disulfide, diallyl trisulfide, diallyl sulfide) and allyl propyl disulfides can be formed by thermally treating garlic and onion whether or not preceded by a enzymatic conversion by ALL. Since these compounds were less abundant in the headspace of PEF + Heat + Mix and Heat + Mix, it can again be concluded that the physical state on which the heat step was applied as well as the degree of tissue disruption might have determined the final abundance.

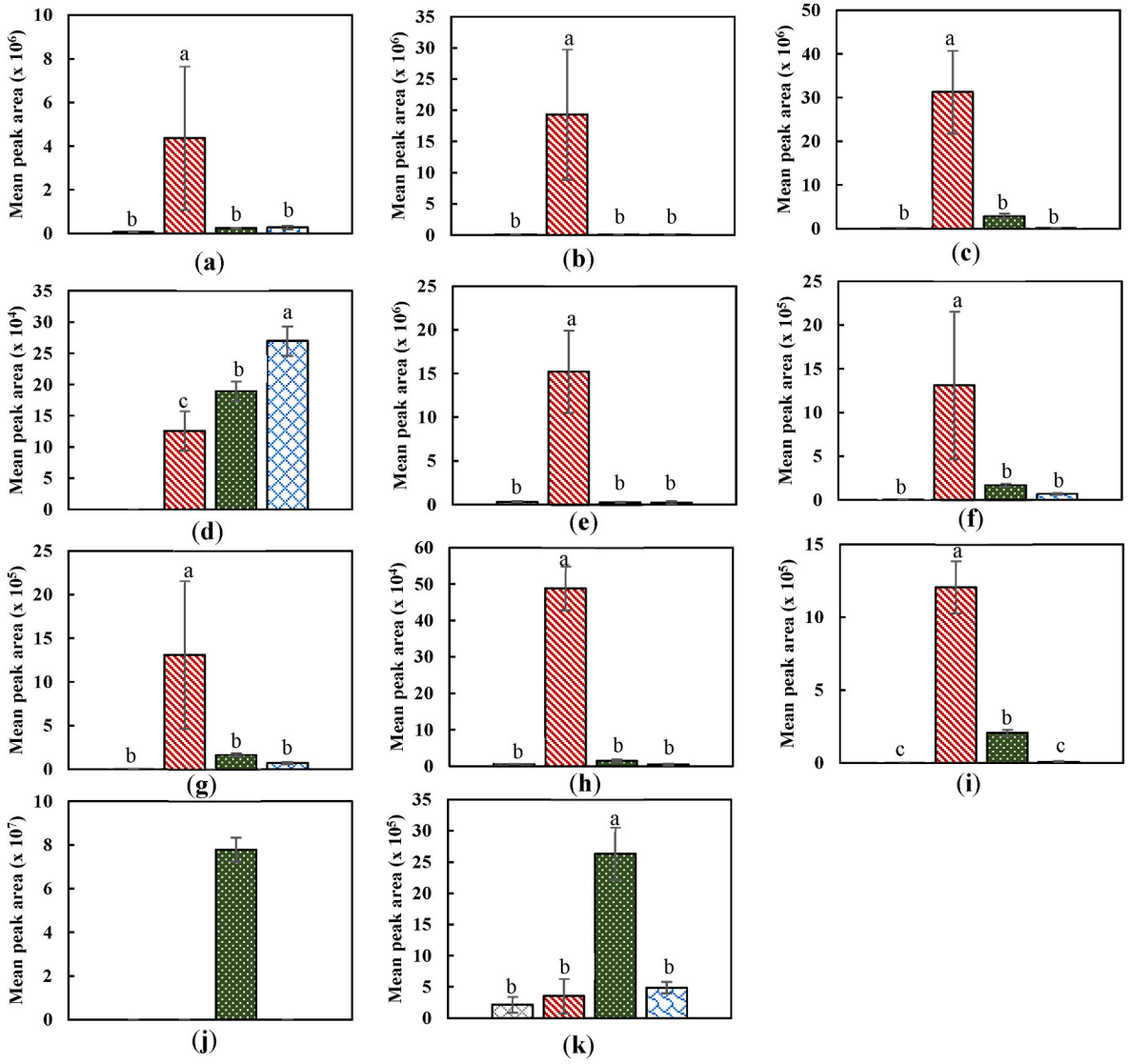

Fig. 3. Specific compound plots of selected discriminant sulfurous compounds in the headspace of different samples ((□) NoPT (■), Mix + Heat (■), PEF + Heat + Mix, and (□) Heat + Mix). (**a**) dimethyl disulfide; (**b**) dimethyl trisulfide; (**c**) methyl propyl trisulfide; (**d**) dimethyl sulfide; (**e**) (E)-1-methyl-2-(prop-1-en-1-yl)disulfide; (**f**) methyl-2-propenyl disulfide; (**g**) methyl-2-propenyl trisulfide; (**h**) (E)-1-allyl-2-(prop-1-en-1-yl)disulfide; (**i**) 1-allyl-3-propyl trisulfide; (**j**) 1-methylethyl propyl disulfide; (**k**) (E)-1-(prop-1-en-1-yl)-3-propyl trisulfide. Different letters indicate significant differences in mean peak area between the samples (*p*-value of 0.05, $N = 12$).

Notwithstanding the fact the aforementioned sulfides mostly seemed to be highly abundant if an intense tissue disruptive step was performed, membrane permeabilization (i.e., partial tissue disruption) as a result of PEF did also induce the ACSOs-ALL reaction pathway as clearly evidenced by the presence of prominent sulfurous volatile compounds such as 1-methylethyl propyl disulfide and (E)-1-(prop-1-en-1-yl)-3-propyl trisulfide (Fig. 3). In the chromatogram of the PEF + Heat + Mix treated sample, a large peak could be observed between retention times 21.05 an 21.25 min (supplementary material). After deconvolution, the peak was separated into several peaks which were all identified as disulfides. A retention time of 21.21 min was linked to 1-methylethyl propyl disulfide, which was moreover selected as a marker, given that match factors were above 80% and the RI matched the RI found in literature (± 50). Even though no clear statements could be set regarding this compound, it is clear that the headspace after a PEF + Heat + Mix treatment was dominated by disulfides, probably arisen as a consequence of the induced reactivity between ACSOs and ALL by PEF. The finding that PEF treatment promotes ACSOs conversion by ALL was previously observed in a study of Nandakumar et al. (2018) in which the impact of PEF on the volatile profile of whole onion was investigated. In the latter study, electric field strengths of 0.3, 0.7, and 1.2 kV/cm, a pulse width of 20 μs, a pulse frequency of 50 Hz and a specific energy of 5 kJ/kg were used for comparison to untreated samples. It was shown that PEF induced membrane permeabilization improving enzyme-substrate interactions. In particular, propanethial-S-oxide, propenyl propyl thiosulfinate, 2-methyl-2-pentenal, dipropyl disulfide, propenyl propyl disulfide, methyl propyl disulfide, and methyl propenyl disulfide were observed in the volatile profile of PEF-treated onion in that study (Nandakumar et al., 2018). The reason why other sulfides are prominently present in the headspace of Mix + Heat compared to PEF + Heat + Mix might be ascribed to the different degree of disruption and the physical state on which the heat was applied, as stated previously, leading to different (abundances of) compounds prone to thermal-induced changes.

3.2.2. *Aldehydes, alcohols and esters*

The specific compound plots of several aldehydes, alcohols and esters are shown in Fig. 4. Pentanal, described in literature as potentially

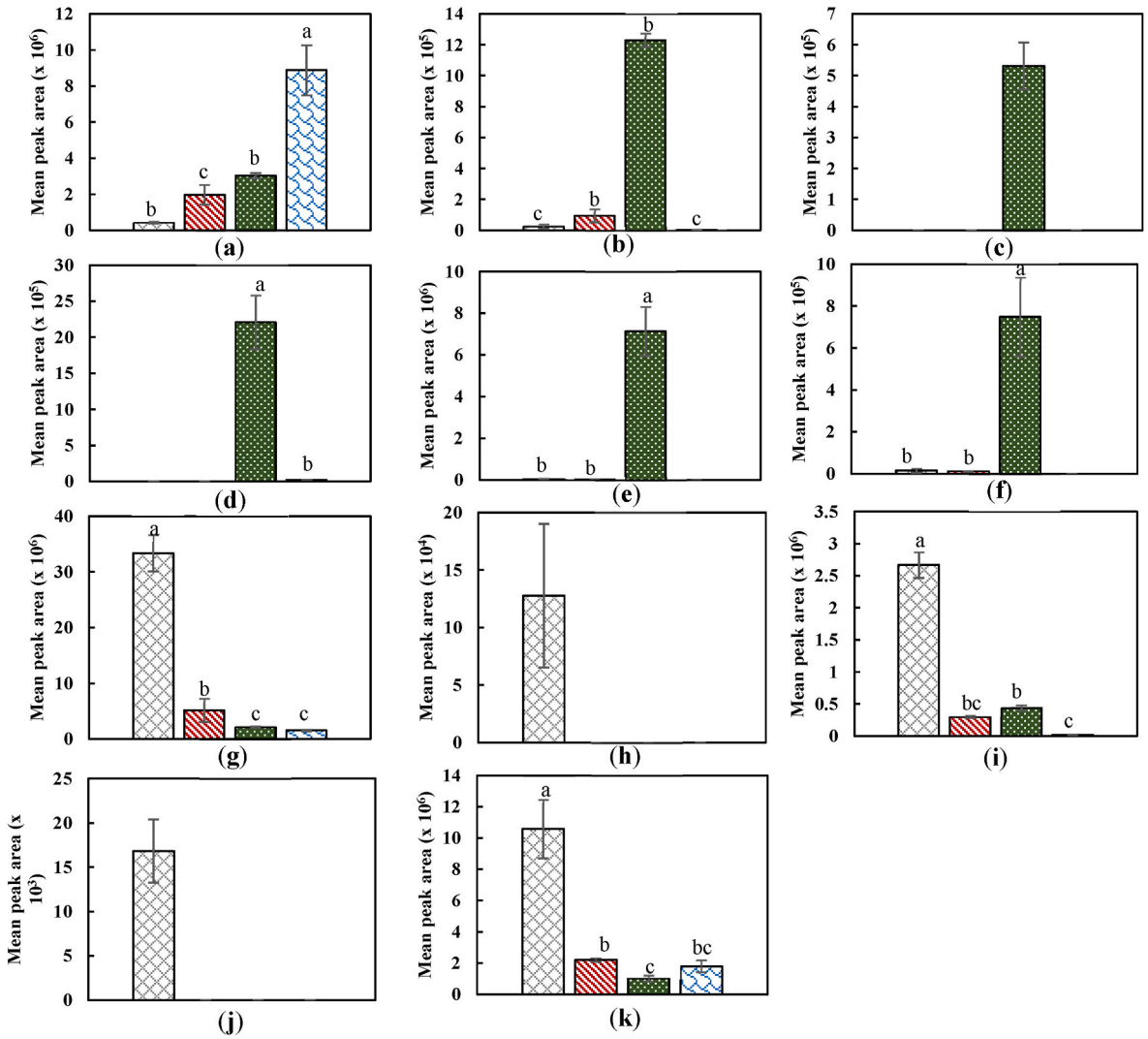

Fig. 4. Specific compound plots of selected discriminant aldehydes, alcohols and esters in the headspace of different samples ((□) NoPT (■), Mix + Heat (■), PEF + Heat + Mix, and (▨) Heat + Mix). (**a**) pentanal; (**b**) (E)-2-methyl-2-butenal; (**c**) (Z)-2-hexen-1-ol, acetate; (**d**) (Z)-3-hexen-1-ol, acetate; (**e**) (E)-2-hexen-1-ol; (**f**) 1-hexanol; (**g**) 2-methyl-2-pentenal; (**h**) propanethial-S-oxide; (**i**) acetaldehyde; (**j**) 3-hexenal; (**k**) hexanal. Different letters indicate significant differences in mean peak area between the samples (p-value of 0.05, $N = 12$).

possessing banana and grassy odor features (Bathgate & Miller, 2019), was particularly noticed in the headspace of the Heat + Mix sample. Its presence can be ascribed to the thermal degradation of substrates and/or thermal degradation products since direct enzyme inactivation was intended during this treatment (Christensen et al., 2007; Wang et al., 2008). This compound could also be present as by-product from an autoxidation reaction (Oyman et al., 2005). The higher amount of pentanal in the headspace after this treatment in comparison with its presence in the headspace of the PEF + Heat + Mix and Mix + Heat samples could be ascribed to the preceding PEF or mix step in the latter treatments, during which possible enzymatic conversion of substrates led to less substrates present to be subjected to thermal degradation. Since pentanal was also observed in the volatile profile of the NoPT sample, this compound was also present in low amount in the raw leek as also reported by Nielsen (2004).

Aldehydes, alcohols and esters particularly observed as being responsible for the distinct volatile profile obtained after PEF + Heat + Mix were (E)-2-methyl-2-butenal, (E)-2-hexen-1-ol, (Z)-2-hexen-1-ol, acetate, (Z)-3-hexen-1-ol, acetate and 1-hexanol (Fig. 4). (E)-2-hexen-1-ol and 1-hexanol are reported in literature as having a green, leaf, walnut, resin and flower odor, dependent on the concentration (Flavornet, 2004). The presence of (E)-2-methyl-2-butenal, which could possibly be perceived as rancid, cooked and oniony (Van Ruth et al., 1995), could most likely be ascribed to the conversion of ACSOs by ALL resulting from the reaction of ethanol, derived from pyruvic acid, with propanal, resulting from propanethial-S-oxide (Nielsen, 2004). The discriminant C6 alcohols and both discriminant C6 acetates observed in the headspace of PEF + Heat + Mix might be the result of the enzyme-catalyzed oxidation of PUFAs by LOX after which the formed hydroperoxides did possibly further react to C6 alcohols and C6 acetates by HPL and ADH and HPL, ADH and AAT, respectively (Engelberth & Engelberth, 2020). Hence, the PEF treatment under current conditions had significant impact on the biochemical reactions indicating the effective permeabilization of the cell membranes separating substrates and enzymes. In addition, the occurrence of these enzymatic reaction pathways is more prominent compared to the contribution from the thermal breakdown of substrates since the headspace after a Heat + Mix treatment, for which both steps were equivalent to the corresponding steps in the PEF + Heat + Mix pretreatment, was not characterized by these volatiles, possibly due to the lack of a prior membrane permeabilization step. In addition, the physical property of the sample on which a heating step was applied was probably determinative for the resulting volatile profile as already previously stated (*cfr.* section 3.2.1). This could possibly be the main explanation why some compounds after

tissue disruption were still present after a PEF + Heat + Mix treatment while undetectable in the headspace of Mix + Heat due to possible (thermal) degradation of concerned compounds.

It is notable that some aldehydes were the dominating compounds in the headspace of the untreated sample (NoPT) (Fig. 4). One main distinctive aldehyde that could be observed in an untreated sample corresponded to 2-methyl-2-pentenal, with a retention time of 13.49 min (supplementary material). This (aliphatic) aldehyde might have arisen from the conversion of *trans*-S-(1-propenyl)-L-cysteine sulfoxide by ALL due to prior slicing of leek stems which could already have triggered some enzymatic reactivities (Resemann et al., 2004; Wang et al., 2008). This compound has previously been described in literature to be a major volatile component detected in onion and can be characterized by green, grassy, herbal, cabbage, paint, prickling, powerful and slightly fruity if its concentration exceeds its sensory threshold (Kebede et al., 2014; Li et al., 2021; Nandakumar et al., 2018; Nielsen et al., 2003; Nielsen & Poll, 2004; Schulz et al., 1998; Wang et al., 2008). Also, the intermediate compound in the ACSOs-ALL pathway, propanethial-S-oxide, characterized by specific odor features like oniony and sweet sulfur as mentioned in literature (Li et al., 2021), possessed discriminative power in the headspace of the NoPT sample. It must be noted that 2-methyl-2-pentenal was also present in the treated samples, probably derived from thermally degraded ACSOs (Li et al., 2021). However, the heat step possibly led to more (thermal) degradation compared to formation (enzymatically or thermally) of this compound, which could explain why this compound was observed to a lesser extent in the treated samples, despite the steered induction of enzymatic conversions during Mix + Heat and PEF + Heat + Mix. Also acetaldehyde was particulary present in the headspace of the NoPT sample, which might indicate its presence in the raw leek and was largely reduced when an additional heat step was applied as indicated by the significantly lower abundance of this compound in the headspace of treated samples. However, a particular amount of this compound in the treated samples might also be present due to ACSOs degradation, as already described in other studies on garlic and onion in which it was shown to be derived from the thermal degradation pathway of S-methylcysteine sulfoxide (MCSO, methiin) and/or alliin which is thermally broken down to α-aminoacrylic acid and hydrolyzed into ammonia and pyruvic acid which is then further decarboxylated to form acetaldehyde (Li et al., 2021; Rössner et al., 2002; Wang et al., 2008). In these samples, also thermal degradation of aldehydes might have contributed to the presence of this compound (Wang et al., 2008). Additionally, this compound might be present as a Strecker aldehyde which can be produced by thermal degradation of alanine (Rainer Cremer, 2000). Furthermore, 3-hexenal and hexanal possessed discriminative power in order to distinguish the headspace of the NoPT sample (Fig. 4). It could be stated that the occurrence of these C6 aldehydes was likely to be derived from the PUFAs-LOX-HPL reaction pathway during which PUFAs (linoleic acid and linolenic acid), significantly present in leek (Nehdi et al., 2020), were cleaved by LOX possibly initiated by previous cutting (Engelberth & Engelberth, 2020; Poltronieri et al., 2018). 3-hexanal, hexanal and acetaldehyde can be featured by green, grassy and/or fresh notes, as reported in literature, if the concentrations exceed threshold values (Bathgate & Miller, 2019; Vincenti et al., 2019). However, C6 aldehydes in high abundance could be perceived as off-flavors (Nielsen et al., 2003). Notable, in the headspace of the treated samples, similar trends for 3-hexenal and hexanal were seen as for the previously described aldehydes, namely that these aldehydes were not or minorly present despite the induced tissue disruption in Mix + Heat and PEF + Heat + Mix and the possible formation of these compounds due to autoxidation (Hammer & Schieberle, 2013). This again was most probably related to the relatively intense subsequent heat step, during which further (enzymatic) conversion of these products and/or thermal degradation could have occurred (Wang et al., 2008).

4. Concluding remarks and future perspective

Based on the outcomes of this study, it can be concluded that targeted pretreatment steps allow to steer (bio)chemical reactions towards specific (flavor-imparting) compounds in leek. In all pretreated samples, the volatile profile is a consequence of both the applied tissue disruptive step (in Mix + Heat and PEF + Heat + Mix) and the heat step in samples that were heat-treated (i.e., in Mix + Heat, PEF + Heat + Mix and Heat + Mix). It could be observed that the level of tissue disruption (i.e., extensive or partial during the mix or PEF treatment, respectively) seemed to impact the identity and/or abundance of volatile compounds meaning that both PEF and mixing did influence (bio)chemical reactions differently. Besides, it was observed that the effect of a heat step was probably also dependent on the physical state of the system on which heating was applied. The volatile profile after Mix + Heat could be distinguished based on the abundant presence of sulfurous compounds, related to the ACSOs-ALL pathway, which could as well be observed in the PEF + Heat + Mix volatile profile but to a much lower extent. The volatile profile after a PEF + Heat + Mix treatment also possessed discriminative aldehydes, alcohols and esters, possibly related to the PUFAs-LOX-HPL pathway. This study showed the potential to steer (bio) chemical reactions in leek which could be used as a starting point for designing processing conditions in order to achieve an intended volatile profile of leek (products) in terms of acceptance and/or preference (if combined with sensory testing in follow-up studies). This approach could be relevant in the context of increasing the consumption of vegetables by humans, since the daily intake is still not met by a large part of the population (Appleton et al., 2016). Moreover, this approach could be extended to all vegetables comprising enzymes and/or substrates that are compartmentalized.

It can be questioned whether different incubation conditions after a Mix or PEF step would intensify the differences in volatile profile of the differently treated samples, given the fact that different endogenous enzymes have different optimal reaction temperatures and taking into account that the subsequent heat step may have affected the presence of the (enzymatically) formed volatile compounds. Therefore, future research could investigate the impact of the incubation temperature of a disrupted system on the resulting volatile profile. In addition, it could be useful to verify whether analytical differences in volatile profile among differently treated samples are also perceived as different by humans by applying discriminative *in vivo* sensory studies.

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CRediT authorship contribution statement

Sophie M. Delbaere: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Project administration. **Tom Bernaerts:** Writing – review & editing. **Flore Vancoillie:** Conceptualization, Methodology, Validation, Writing – review & editing. **Carolien Buvé:** Conceptualization, Methodology. **Marc E. Hendrickx:** Conceptualization, Validation. **Tara Grauwet:** Conceptualization, Validation, Resources, Funding acquisition, Supervision. **Ann M. Van Loey:** Conceptualization, Validation, Resources, Writing – review & editing, Project administration, Funding acquisition, Supervision, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare to have no known financial interests or personal relationships with other people or organizations that could have influenced the current work.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.114205.

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