The volatile profile of pasteurized leek (*Allium ampeloprasum* var. *porrum*) and Brussels sprouts (*Brassica oleracea* var. *gemmifera*) (products), as a witness to (bio)chemical reactivity, influenced by pretreatment and successive refrigerated storage

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Abstract

Processing can affect (bio)chemical conversions in vegetables and can act on their volatile properties accordingly. In this study, the integrated effect of pretreatment and pasteurization on the volatile profile of leek and Brussels sprouts and the change of this profile upon refrigerated storage were investigated. Pretreatments were specifically selected to steer biochemical reactivities to different extents. Volatile profiles were analyzed by headspace-solid phase microextraction-gas chromatography-mass spectrometry. For both vegetables, it was observed that different pretreatments prior to a pasteurization step led to diverse volatile profiles. The differences in volatile profiles observed in the different samples were presumably attributed to the different degrees of enzymatic conversions, further conversions of enzymatically formed products and thermally induced reactivities. Interestingly, the observed initial relative differences between volatile profiles of differently pretreated pasteurized samples were still observed after a refrigerated storage of 4 weeks at 4 °C. In conclusion, refrigerated storage only limitedly affected the resulting volatile profile.

Keywords

leek, Brussels sprouts, flavor, processing, preservation, refrigerated storage

Abbreviations

alcohol acetyl transferase: AAT; alliinase: ALL; Automated Mass Spectral Deconvolution and Identification System: AMDIS; cystin (sulfoxide) lyase: C-S lyase; divinylbenzene/carboxen/polydimethylsiloxane: DVB/CAR/PDMS; electron ionization: EI; epithiospecifier protein: ESP; glucosinolates: GSLs; headspace-solid phase microextraction-gas chromatography-mass spectrometry: HS-SPME-GC-MS; hydroperoxide lyase: HPL; incubation: Inc; latent variables: LVs; lipoxygenase: LOX; Mass Profiler Professional: MPP; mass selective detector: MSD; MVDA: multivariate data analysis; myrosinase: MYR; no pretreatment: NoPT; Partial Least Squares Discriminant Analysis: PLS-DA; pasteurization: Past; peroxidase: POD; poly unsaturated fatty acids: PUFAs; Principal Component Analysis: PCA; retention index: RI; Root Mean Squared Error of Cross Validation: RMSECV; S-Alk(en)yl-L-cysteine sulfoxide: ACSOs; Variable iIdentification cCoefficients: VIDs.

1. Introduction

Pasteurization is a frequently applied thermal preservation treatment guaranteeing safe products by inactivating spoilage microorganisms. However, due to the relatively mild processing conditions, bacterial spores and thermotolerant spoilage microorganisms can tolerate the pasteurization intensities, thus, growth of aforesaid organisms may occur which determines the safety and microbial stability level of the food product. Therefore, the pasteurization process is usually followed by refrigerated storage, ensuring safe food products, especially if the food has pH values greater than 4.6 and water activities higher than 0.92 (Aamir et al., 2013; Silva & Gibbs, 2012).

Despite the positive impact from a safety point of view, thermal processes such as pasteurization - as well as the consecutive storage - can lead to a conceivable (negative) impact on the quality of food products. This decrease in quality can affect the acceptance and, consequently, the consumption behavior towards (stored) food products, even if the product is safe (Aamir et al., 2013; Ávila & Silva, 1999; Barrett et al., 2010; Gonçalves et al., 2007; Shen et al., 2018). Thermal processing (and storage) can impact, on the one hand, the nutritional quality by possible nutrient degradation and, on the other hand, the sensorial quality, by for instance the degradation of color and texture and changes in the flavor characteristics, comprising both aroma and taste, for instance by the formation of off-flavors (e.g., sulfurous, bitter notes) (Aamir et al., 2013; Ávila & Silva, 1999; Barrett et al., 2010; Koutidou et al., 2017; Vervoort et al., 2012; Wang et al., 2008). Formation of off-flavors in heat-treated vegetables and initiated by storage has already been addressed in literature (Wang et al., 2008).

The presence of volatile compounds (and possible off-flavors) in food is a witness of the occurrence of both non-enzymatic reactions (e.g., heat-induced (degradation) reactions, Maillard reactions, autoxidation) and enzymatic reactions (i.e., biochemical reactions) (Bones & Rossiter, 2006; Christensen et al., 2007; Resemann et al., 2004; Rössner et al., 2002). Notwithstanding the stated possible impact of a pasteurization process and subsequent storage on the quality of a product (i.e., loss of quality), this quality loss is believed to take place to a limited extent since temperatures used during a pasteurization process can trigger besides microbial inactivation also enzyme inactivation preventing enzyme-catalyzed conversions. The lower occurrence of the

latter can prevent possible formation of compounds that (negatively) affect the quality of the product (during later storage) (Aamir et al., 2013; Peng et al., 2017; Silva & Gibbs, 2012).

Besides preservation (e.g., pasteurization) processes and storage, also specific pretreatments, as part of the food processing chain and typically implemented to control biochemical (i.e., enzymatic) reactivities, can be carried out (prior to a possible preservation and storage step) and convey effects on the (initial) food quality (Singh et al., 2019; Zhang et al., 2021). In *Allium* vegetables, such as leek (*Allium ampeloprasum* var. *porrum*), the main enzymatic reaction pathway that contributes to the distinctive aromatic characteristics is the conversion of non-protein sulfur-containing amino acids, namely *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs), catalyzed by the enzyme alliinase (ALL) (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; Resemann et al., 2004; Rössner et al., 2002; Wang et al., 2008). These substrates (i.e., ACSOs) can also be present in cruciferous plants such as Brussels sprouts (*Brassica oleracea* var. *gemmifera*) for which the reaction is mediated by the enzyme cystin (sulfoxide) lyase (C-S lyase) (EC. 4.4.1.10) (Rössner et al., 2002; Tulio, et al., 2002). ACSOs are precursors of various (flavor-imparting) secondary sulfurous compounds as a result of their conversion by an α,β-elimination, resulting in pyruvic acid, ammonia and alk(en)yl sulfenic acids. The latter can further degrade into thiosulfinates which rearrange to the aforesaid secondary sulfurous compounds (Lee et al., 2009; Nielsen et al., 2003). The most prominent enzymatic reaction pathway that leads to volatile compounds in Brussels sprouts is the hydrolysis of β-thioglucoside-N-hydroxysulfates, namely glucosinolates (GSLs), catalyzed by the enzyme myrosinase (MYR) (EC 3.2.1-3.2.3). GSLs are non-volatile sulfur-containing precursors causing the formation of (flavor-affecting) bio-active breakdown products such as isothiocyanates, thiocyanates and nitriles, for which the identities and abundances are regulated by several extrinsic factors (e.g., temperature, pH) and the presence of cofactors (e.g., epithiospecifier protein (ESP)) (Bricker et al., 2014; N. Frank et al., 2010; Kelly et al., 1998; Kissen et al., 2009; Oliviero et al., 2018; Ortner & Granvogl, 2018; Pecháček et al., 1997; Tian et al., 2005; Wieczorek et al., 2018). Besides, a prominent pathway in both vegetables is the conversion of lipid compounds (i.e., poly unsaturated fatty acids (PUFAs)) by lipoxygenase (LOX) (EC.1.13.11.12) followed by the action of hydroperoxide lyase (HPL) (EC. 4.2.99.-) and alcohol acetyl transferase (AAT) (EC 2.3.1.84). The latter pathway is an oxidation pathway that generates (C6 and C9) aldehydes, alcohols and esters which are liable to further isomerization and

breakdown and can result in off-flavors when present in increased concentrations (Christensen et al., 2007; Engelberth & Engelberth, 2020; D. Frank et al., 2018; Koutidou et al., 2017; Nielsen et al., 2003; Van Boekel, 2008; Vincenti et al., 2019). The aforementioned reactions are achieved by tissue disruption, enabling the formation of various (possible flavor-imparting) volatile compounds due to rupture of the existing compartmentalization in the plant cell, separating both substrate and enzyme (Christensen et al., 2007; D. Frank et al., 2018; Kissen et al., 2009; Li et al., 2021; Nielsen et al., 2003; Resemann et al., 2004; Tian et al., 2005; Van Eylen et al., 2008; Wang et al., 2008; Zhao et al., 2014). More specifically, while ACSOs are located in the cytoplasm of the plant cell, ALL is present in the vacuole (Nielsen et al., 2003; Wang et al., 2008). As for cruciferous plants, MYR is stored in the cytoplasm of specialized myrosin cells, whereas S-cells are storage sites for GSLs (Pan et al., 2022; Shirakawa & Hara-nishimura, 2018). LOX can be located in several plant cell compartments of the plant cell such as in lipid bodies, in chloroplasts, in the vacuole or in the cytoplasm, while HPL is reported to be membrane-bound (Vincenti et al., 2019).

Many studies have already been conducted focusing on the impact of one (or two) processing step(s) on the quality of food. However, investigating and comparing the integrated effect of different combined steps in the food processing chain (e.g., combining pretreatment, preservation and storage) on the volatile profile of leek and Brussels sprouts is, to the best of our knowledge, non-existing. Nonetheless, this is interesting to investigate due to the hypothesized different effects of different combinations of steps on the (bio)chemical conversions in vegetables of two different plant families and on the resulting volatile characteristics. This is of outmost relevance since not one but different sequences of steps are commonly applied in industry. Based on research conducted by Delbaere, Bernaerts, Vancoillie, et al. (2022) and Delbaere, Bernaerts, Vangrunderbeek, et al. (2022), it was shown that pretreatments can steer (bio)chemical conversions in leek and Brussels sprouts, respectively, and as such impact the concomitant volatile profile. In this context, the question arises whether those different volatile profiles are still observed after different pretreatments followed by an additional pasteurization step and if subsequent refrigerated storage has an impact on the relative observed differences. In addition, the effect of refrigerated storage on the volatile characteristics of pasteurized leek and Brussels sprouts (products) would be of relevance to explore.

Therefore, this integrated approach will be investigated for leek and Brussels in this study. First, the effect of pretreatment on the volatile profile of pasteurized leek and Brussels sprouts will be elucidated. Moreover, it will also be clarified what effect a refrigerated storage for 4 weeks at 4 °C has on the differences observed in the volatiles profiles of differently pretreated pasteurized leek and Brussels sprouts. Second, volatile changes upon refrigerated storage after pasteurization are further explored for differently pretreated leek and Brussels sprouts, with particular focus on the changes in specific volatile compounds during storage. Pretreatments will include mixing, enabling extensive tissue disruption, and mixing followed by incubating, presumed to further enhance enzymatic conversions. Also, a chopped (for leek)/intact system (for Brussels sprouts) will be taken with before pasteurization, hypothesized to impart enzymatic conversions minimally. Volatile profiles will be analyzed by headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS). Special attention will be paid to gaining deeper insight into the underlying reactivities that could have occurred during (pre)treatment, witnessed by the analyzed volatile profiles.

2. Materials and methods

2.1 Experimental set-up

An overview of the experimental set-up is given in **Figure 1**. All pasteurization cycles were designed beforehand in order to achieve a P-value of 11-14 min (z-value: 10 °C, T_{ref}: 90 °C) in the coldest spot of a package.

2.2 Preparation, processing and storage of vegetables

2.2.1 Purchase of raw material

Raw leek (*Allium ampeloprasum* var. *porrum*) and Brussels sprouts (*Brassica oleracea* var. *gemmifera*) were purchased on the day of harvesting and were used for all processing steps within 1 week after purchase. Until processing, the samples were stored in a cooling room of 3 °C. Leek, cv. Belton, was harvested in November 2021, originated from Koolskamp, Belgium and matched a ratio of 40:60 (white:green). Brussels Sprouts, originating from Nieuwkerke, Belgium were purchased in November 2021, were from the Sofia variety and had diameters ranging from 15-25 mm. The aforementioned physical parameters were selected given their industrial relevance.

2.2.2 Pretreatments

2.2.2.1 No Pretreatment (NoPT)

Leek and Brussels sprouts were cleaned with tap water and tapped dry afterwards. Damaged leaves of leek and very small, dirty, or damaged Brussels sprouts were discarded. Subsequently, leek was chopped into snips of approximately 5 x 1.5 cm. Brussels sprouts were kept whole. The vegetables were then divided over vacuum bags (165 x 230 mm, PET/ALU/NY/LDPE) consisting of about 150 g leek snips and 150 g Brussels sprouts each and were vacuum packed afterwards.

2.2.2.2 Mix

Cleaned and snipped leek and Brussels sprouts were mixed for 1 min in a Thermomix (at 10,700 rpm) with cold demineralized water (3:2 (w: v) for leek, 1:1 (w: v) for Brussels sprouts). The latter steps were repeated and the obtained puree was pooled. The resulting puree was divided over dark vacuum bags containing about 200 g each and were vacuum packed.

2.2.2.3 Mix + Incubation (Mix + Inc)

Samples were prepared similarly as described in section 2.2.2.2. In addition, an incubation (Inc) step for 90 min at 40 °C, hypothesized to enable further biochemical conversions induced by mixing, was added which took place in a pilot-scale water-cascading retort autoclave (Barriquand Steriflow, Paris, France).

2.2.3 Pasteurization (Past) + storage

Prepared bags (*cfr.*, 2.2.2.1, 2.2.2.2 and 2.2.2.3) were placed vertically on a rack (i.e., hanging) which was transferred to a pilot-scale water-cascading retort (Barriquand Steriflow, Paris, France). In order to monitor the temperature during treatment, two bags were provided with thermocouples at the coldest spot. The samples were subjected to a pasteurization (Past) process under conditions according to **Table 1**. The pasteurization cycle was verified via a qualitative peroxidase (POD) test to guarantee POD negative activity, which is considered the most heat stable enzyme in vegetables (McLellan & Robinson, 1981). Subsequently, the bags were stored in a fridge of 4 °C for a maximum of 4 weeks. At 11 predetermined time points during refrigerated storage for 4 weeks at 4 °C, for each system, one bag was transferred to a −40 °C freezer until analyses, a temperature lower than the glass transition temperature of both vegetables (data not shown). As for the chopped leek/ intact

Brussels sprouts, at the specified time points, the vegetables were mixed with cold demineralized water (3:2 (w: v) for leek, 1:1 (w: v) for Brussels sprouts) in order to obtain a puree-like system necessary for analytical purpose. The resulting samples are referred to as NoPT + Past, Mix + Past and Mix + Inc + Past.

2.3 Analysis of the volatile profile

2.3.1 Sample preparation

Samples were thawed in a standardized way on the day of analyses in a water bath at 25 °C for 20 min. 0.8 g of sample was put into a 10 mL amber glass vial (VWR International, Radnor, PA, USA) together with 3 mL of saturated NaCl solution and 0.2 mL of demineralized water. Each of the vials were tightly closed using metal screw-caps with a PTFE/silicone septum seal (Grace, Columbia, MD, USA). For each system, six replicates were analyzed which was predetermined based on a replicates test (data not shown). An amount of 100 µL internal standard solution (diluted 3-heptanone solution) was added to each vial using a gastight syringe in order to enable the detection of potential fluctuations in the signal and to follow-up the operational behavior of the analytical system. Fiber degradation, monitored by adding control samples in each sequence, did not occur.

2.3.2 Headspace-solid-phase-microextraction (HS-SPME-GC-MS)

A HS-SPME-GC-MS method was used to analyze the volatile profiles and was based on the method described by Kebede et al. (2014) (Kebede et al., 2014). The prepared vials were homogenized and transferred to the cooling tray (at 10 °C) of the CombiPal autosampler (CTC Analytics AG, Zwingen, Switzerland). Samples were analyzed by an untargeted approach using headspace fingerprinting technique which was conducted 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). Different parameters of the GC-MS analyses were optimized beforehand. The incubation time under an agitation speed of 500 rpm was 8 min at 40 °C. Next, the volatile components present in the headspace were extracted for 20 min at 40 °C using a 30/50 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (StableFlex, Supelco, Bellefonte, PA, USA). The fiber was preconditioned prior to extraction according to the manufacturer guideline. At 230 °C, the volatiles were desorbed for 2 min at the injection port of the GC. Consequently, the desorbed

volatiles were separated on an HP Innowax column, 60 m x 0.25 mm i.d., 250 µm film thickness (Agilent Technologies J&W, Santa Clara, CA, USA). Helium (purity ≥ 99.9999%) with a constant flow of 1.273 ml/min was used as a carrier gas. When injection in a split mode (1:5) in the oven was completed, a specific oven program was followed with a starting temperature of 40 °C for 2 min, 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 before cooling back to 40 °C. The temperature of the ion source and quadrupole amounted 230 °C and 150 °C, respectively. Mass spectra were obtained by electron ionization (EI) mode at 70 eV in scanning mode (m/z 35-400) at 3.9 scans/s. Samples were randomly analyzed.

2.4 Multivariate data analysis

In a first step, Automated Mass Spectral Deconvolution and Identification System (AMDIS) software (Version 2.72, 2014, National Institute of Standards and Technology, Gaithersburg, Maryland, USA) allowed pre-processing the volatile data by deconvoluting the peaks. Using this software, pure component spectra were extracted from the complex chromatograms. The latter software also enabled to build a retention index (RI) calibration file which was obtained by running homologous series of C8-C20 alkane standards which were analyzed under the used GC-MS conditions. This calibration file was used for data compound identification which was at the same time executed by comparing the deconvoluted mass spectra with the reference spectra found in the spectral library of NIST (NIST14, version 2.2, National Institute of Standards and Technology, Gaithersburg, MD, USA). As a second step, Mass Profiler Professional (MPP) software (version B12.00, 2012, Agilent Technologies, Diegem, Belgium) enabled peak filtering, alignment and baseline correction of the deconvoluted data. The result was a spreadsheet containing peak areas for every peak detected in each sample. This data table comprised the X-variables in the columns (i.e., volatiles) and the Y-variables in the rows (i.e., type of treatment or storage time). In what follows, data sets combining the volatile data of differently pretreated pasteurized leek or Brussels sprouts are referred to as 'categorical Y data sets' whereas data sets combining the volatile data of differently stored (in the context of time of storage) pasteurized pretreated leek or Brussels sprouts products are cited as 'the continuous Y data sets'. Thirdly, Solo software (Version 8.7.1, 2020 Eigenvector Research, Wenatchee, WA, USA) allowed multivariate data analysis. In a first instance, pre-processing was performed to mean-center the data and to give the data equal variance by weighing the variables

by their standard deviation. In a second instance, Principal Component Analysis (PCA) was conducted on the data sets as an exploratory modelling technique to screen the data for potential outliers. In a third instance, the effect of pretreatment on the resulting volatile profile of pasteurized leek or Brussels sprouts in the beginning of storage was investigated. Also, it was uncovered if this effect was still observed in the final stage of the refrigerated storage period. Therefore, a Partial Least Squares Discriminant Analysis (PLS-DA), which is a regression based classification method, was built with the categorical Y data sets based on latent variables (LVs), aiming to minimize the covariance between the X-variables (i.e., volatiles) and the categorical Y-variables (i.e., differently pretreated, pasteurized samples/groups/classes) in the model calculated. On the contrary, the continuous Y data sets were subjected to PLS regression to evaluate the changes in volatile profiles of differently pretreated pasteurized products upon refrigerated storage. In this modelling technique, LVs are linear combinations of the volatiles representing the X-variables for which the trend as function of storage time (Y-variable) is maximally explained. Complexities of the models were determined based on lowest value of the Root Mean Squared Error of Cross Validation (RMSECV). Moreover, it was ensured the number of LVs that was chosen to build the model did not exceed the number of classes/groups taken with in comparison.

A graphical representation of the differences in volatile profiles as affected by pretreatment or storage time can be given in biplots, which combine correlation loadings plots and scores plots. All biplots were constructed in OriginPro 8 (Origin Lab Corporation, Northampton, MA, USA). In order to quantitatively investigate which volatiles are responsible for the distinct behavior in volatile profiles between the groups being compared, in case of the PLS-DA models, or in order to describe the evolution in volatile profiles upon storage, regarding the PLS regression models, Variable identification coefficients (VIDs) were calculated for each of the volatile compound for each of the groups (i.e., differently pretreated pasteurized samples/different storage times) and represent the correlation coefficients between each original X-variable and the Y-variable(s) as estimated by the model. Variables with VIDs with absolute values above 0.9 and 0.7, calculated after PLS-DA (on categorical Y data sets) and PLS regression (on continuous Y data sets), respectively, were considered of interest and were referred to as discriminant volatiles (markers). Since the effect of processing was hypothesized to be higher compared to the effect of storage, the amount of markers was expected to be higher for categorical Y data sets. Therefore, the VID thresholds were set differently for the categorical Y data sets and the continuous Y data sets

(i.e., higher for categorical Y data sets). This hypothesis was confirmed after analyzing the data, where it was observed the amount of markers comprising a $|VID| \geq 0.9$ for the continuous Y data sets was almost none. Discriminant compound plots of selected relevant fingerprinting markers were plotted representing the mean peak area of the compound as a function of treatment or as a function of storage time. Identity of the markers was confirmed by comparing their RI with the value found in literature. Components of which the RI was not found in literature or did not match the value found in literature are indicated as 'tentatively identified' and 'unidentified', respectively. Moreover, a threshold match of 80% was taken into consideration for identification.

2.5 Statistical analyses

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 Effect of pretreatment on the volatile profile of (cold stored) pasteurized vegetables

3.1.1 Leek

3.1.1.1 Graphical representation and identification of volatile compounds in pretreated and pasteurized leek

In the beginning and at the end of the refrigerated storage period, 114 and 100 components, respectively were observed in the chromatograms of all pretreated, pasteurized (and stored) leek samples. To describe the data of the first day of the storage period, a PLS-DA model was constructed with 3 LVs, explaining 98.30% of the total Y-variance for which the first 2 LVs accounted for 92.77%. Given this relatively high percentage, it was chosen to only depict the biplot of LV2 as a function of LV1 (**Figure 2a**). For the categorical Y data set at the end of storage, 2 LVs were used to construct a PLS-DA model, explaining 97.71% total Y-variance and the biplot of LV2 as a function of LV1 is presented in **Figure 2b**. Arrows shown in the biplot point to the different classes (groups/pretreatments) for which the angle between the arrows is an important parameter as it describes how samples relate to each other based on their volatile profile (Vervoort et al., 2012). As it could be observed in the biplots (**Figure 2**), the three different pretreatments clearly led to different volatile profiles after pasteurization which evidently demonstrates that varying processing steps/conditions during pretreatment can be used to steer

(bio)chemical conversions/reactivities in leek. The observation that pretreatment can steer biochemical conversions in leek has also been shown previously by Delbaere, Bernaerts, Vancoillie, et al. (2022) (Delbaere, Bernaerts, Vancoillie, et al., 2022). In the latter study, several pretreatments, specifically designed to induce and/or minimize biochemical conversions, were implemented on leek (i.e., mixing + heating, heating + mixing, pulsed electric field treatment + heating + mixing), and were observed to cause distinctive volatile profiles. The results obtained in the current study show that with an additional pasteurization step after pretreatment, the distinctive impact of different pretreatments on the volatile profiles is still observed. . Additionally, the observed differences in the headspaces are shown to be only slightly impacted upon increased refrigerated storage time, as can be deduced from the similar relative position of the groups . It was verified that the discriminatory behavior was reflected in the presence of similar markers in the beginning and on the last day of the refrigerated storage (data not shown). This shows that the effect of storage on the presence/abundance of specific volatile compounds seemed to be inferior compared to the effect of pretreatment. **Table 2** lists the VID, identity, chemical class and RI of the discriminant compounds at the beginning of the storage period.

3.1.1.2 Interpretation of the identity and relative abundance of selected discriminant compounds in pretreated pasteurized leek

To compare the volatile profiles of differently pretreated and pasteurized leek and to gain insight into possible reaction pathways that took place during the applied treatments, several discriminant compounds can be selected. The selection was based on the distinguishable behavior of the corresponding compound and its possibility to link its presence to specific important quality-relating (bio)chemical reaction pathways. However, it is sometimes challenging to unequivocally ascribe a compound to a specific reaction pathway, since several consecutive/combinations of reactions could have taken place during the applied treatments. More specifically, extensive tissue disruption (by mixing) was performed aimed to induce biochemical conversions, hypothesized to be more pronounced when followed by an incubation step. On the contrary, NoPT (chopped) leek was included for which only minimal enzyme-substrate conversions were expected. Moreover, given the implementation of a pasteurization step in all treatments, also heat-induced reactivities (i.e., non-enzymatic reactivities) could have occurred which will also be taken into consideration in relating compounds to specified reaction pathways.

Discriminant compound plots of selected important quality-related discriminant aldehydes and alcohols, on the one hand, and sulfurous compounds, on the other hand, are depicted in **Figure 3**. It was chosen to represent bar plots as a summation of all compounds belonging to one chemical class. As such, the relative abundances of compounds belonging to specific chemical classes in the headspaces can be easily visualized and compared between different treatments.

In what follows, individual compounds as displayed in **Figure 3** will be discussed in more detail. **Hexanal**, **(E)-2-hexenal, (Z)-2-hexenal** and **2-methyl-2-pentenal** were less present in the headspace of the NoPT + Past sample (VID < −0.9) in the beginning of the refrigerated storage (**Table 2**). As for hexanal, (E)-2-hexenal and 2-hexenal, besides the possible formation of these compounds via enzymatic oxygenation of PUFAs by LOX, after which the resulting hydroperoxide is converted by HPL, it could be postulated that the presence of these compounds can also be ascribed to the non-enzymatic autoxidation of linoleic acid (Cao et al., 2014; Engelberth & Engelberth, 2020; Vincenti et al., 2019). Furthermore, the abundances of these compounds might also be related to the thermal formation of hexanal, (E)-2-hexenal, (Z)-2-hexenal and 2-methyl-2-pentenal by thermal degradation of PUFAs or the thermal degradation of hexanal, (E)-2-hexenal, (Z)-2-hexenal and 2-methyl-2-pentenal into other compounds. Thermally induced substrate conversion leading to the aforementioned aldehydes, however, is expected to take place minorly since otherwise, abundances would also be high in the chopped samples. Indeed, since no extensive enzyme inductive step (e.g., mixing) was implemented during sample preparation (Grebenteuch et al., 2021), chopped samples are believed to still have more quality-related substrates (i.e., PUFAs) left before pasteurization prone to be affected by heat. The fact that hexanal, (E)-2-hexenal and (Z)-2-hexenal are more representative in the headspaces of the Mix + Past and Mix + Inc + Past samples in comparison to their abundances in the headspace after NoPT + Past, supports the hypothesis that enzymatic conversion is more induced when extensive tissue disruption was implemented, compared to the NoPT sample, in which enzymatic conversions were intended to take place to a limited extent (**Figure 3**). Moreover, enzymatic conversions (to other compounds) might have been further induced by the subsequent steps during or before (incubation and) pasteurization. **2-methyl-2-pentenal** is a well-known conversion product of trans-S(-1-propenyl)-L-cysteine sulfoxide mediated by ALL arisen as a self-condensation and subsequent dehydration product of two molecules of propanal (Nandakumar et al., 2018; Wang et al., 2008).

Notwithstanding its relatively low presence over all aldehydes, the significantly higher abundance of this compound in both mixed samples can again be addressed to the extensive disruptive mixing step, enabling decompartmentalization of the cell organelles enclosing ACSOs, LOX and HPL facilitating their interaction leading to 2-methyl-2-pentenal (Nandakumar et al., 2018; Resemann et al., 2004). The presence of **benzaldehyde** might be a result of the phenylalanine (i.e., an amino acid) degradation (via phenylacetaldehyde) by Strecker degradation which comprises an oxidative deamination and decarboxylation of α-amino acids and occurs in presence of dicarbonylic compounds formed during Maillard reactions (Adamiec et al., 2001).

Unexpectedly, as seen in **Figure 3**, no significantly higher abundance of **hexanal**, **(E)-2-hexenal, (Z)-2-hexenal** (and **2-methyl-2-pentenal)** could be observed in the volatile profiles after the mix treatment followed by an incubation step, which does not match the expectations since by incubation, more enzymatic conversions and thus presence of these enzymatic reaction products were expected. Several possible explanations can be given to elucidate this contradiction. First, it could be postulated that incubation possibly did lead to more biochemical reactivities, but that the aforesaid aldehydes might have been further converted to other components during incubation and/or pasteurization during the Mix + Inc + Past treatment (Zamora et al., 2015). In this context, it can be stated that notwithstanding the abundance of aldehydes in both mixed samples is observed to be similar, their occurrence can possibly be linked to other (bio)chemical reactions. More specifically, it is expected that at the level of the pasteurization step, more substrates (i.e., PUFAs) are presumed to still be present in the Mix sample that was not incubated, prone to other (bio)chemical reactions as those substrates initially present in the incubated mixed sample (Wang et al., 2008). Second, it could be that during both treatments including a mix step, all PUFAs have already reacted away before (incubation and) pasteurization took place and therefore results in no net difference between the abundance of aldehydes in both mixed samples. The latter explanation might be the most plausible since ACSOs were observed to be reacted away almost completely before pasteurization (data not shown). This might probably be also valid for PUFAs, since HPL is membrane-bound and thus, PUFAs are probably even more susceptibly for enzymatic conversion compared to ACSOs (Vincenti et al., 2019).

For certain **trisulfides (i.e., dimethyl trisulfide and methyl 2-propenyl trisulfide)**, a positive VID was ascribed to those compounds in the volatile profile after a Mix + Past treatment, while this value was negative

for those compounds in the volatile profile after a NoPT + Past treatment. On the one hand, enzymatic conversion of ACSOs by ALL could result in the formation of these trisulfides as a result of extensive tissue disruption (Nandakumar et al., 2018). Dimethyl trisulfide has been reported earlier in literature to be part of the most important odors detected in freshly cut leek (Nielsen & Poll, 2004). In the volatile profile after the NoPT + Past treatment, chopping (i.e., partial tissue disruption) the leek could have initiated this reaction pathway while mixing caused an extensive tissue disruption which can explain the higher abundance of these compounds in the volatile profile after mixing since in the latter samples, interaction between ACSOs and ALL was more easily allowed (Nielsen et al., 2004). These reactions could have further taken place during the subsequent (incubation and) pasteurization. On the other hand, thermal effects could have influenced the abundance of those sulfides for instance by the thermal degradation of ACSOs leading to trisulfides or further thermal degradation of these trisulfides to other compounds. Thermal effects have already been shown to exert an influence on the presence of **dimethyl trisulfide** as reported to be a product derived from the thermal degradation of methyl cysteine sulfoxide in garlic and onion treated at 100-120 °C for 60 min (Li et al., 2021). The fact that a lower abundance for this compound was observed in the volatile profile after Mix + Inc + Past treatment was again not in line with the hypothesis of extra formation due to extended incubation. Similar plausible reasons for this observation as for the aforementioned aldehydes (i.e., **hexanal**, **(E)-2-hexenal, (Z)-2-hexenal** (and **2-methyl-2-pentenal**) can be set in this respect.

Markedly, as can be seen in **Figure 3**, as opposed to the aforementioned aldehydes and sulfurous compounds, different trends were observed for **pentanal** and **(E)-1-(prop-1-en-1-yl)-3-propyltrisulfide,** which were observed to be more abundant in the headspace of the NoPT + Past sample. This can presumably be ascribed to the thermal degradation of substrates (i.e., PUFAs and/or ACSOs), which were still relatively abundantly present after a non-extensive tissue wounding (i.e., chopping), leading to these compounds (Wang et al., 2008) Contrarily, PUFAs and ACSOs in the mixed samples are presumably already converted enzymatically to a larger extent. Besides thermal formation, the abundance of **pentanal** and **(E)-1-(prop-1-en-1-yl)-3-propyltrisulfide** in the NoPT + Past sample could also be a result of the enzymatic conversion induced by chopping (Wang et al., 2008). As stated before, PUFAs and/or ACSOs, prone to be subjected to thermal degradation, might still be significantly present in the mixed sample lacking a following incubation before pasteurization (Zamora et al.,

2015). However, thermal degradation of substrates leading to **pentanal** and **(E)-1-(prop-1-en-1-yl)-3-propyltrisulfide** in the Mix + Past sample seems to not have happened as observed in the lower abundance of these compounds. This might signify that the physical state of the system, subjected to the heat of the pasteurization process, might have been another determinative factor in defining the final abundance of the resulting volatile compounds (Delbaere, Bernaerts, Vancoillie, et al., 2022). Moreover, as stated before, since it was observed that ACSOs were almost fully reacted away in the mixed samples (data not shown), it might be more plausible that ACSOs and/or PUFAs might already have been converted before the pasteurization was initiated (Wang et al., 2008).

3.1.2 Brussels sprouts

3.1.2.1 Graphical representation and identification of volatiles in pretreated pasteurized Brussels sprouts

The amount of volatiles observed over all chromatograms of pretreated, pasteurized (and stored) Brussels sprouts in the beginning and at the end of the refrigerated storage was 117 and 123, respectively. In the beginning and on the last day of storage, a total of 2 LVs were selected to build the PLS-DA model, accounting for 99.37 and 99.56% of the total Y-variance, respectively. Similarly as for leek, the different pretreatments led to different volatile profiles, exemplifying that processing conditions can be used to steer (bio)chemical conversions in Brussels sprouts, as also demonstrated in the study of Delbaere, Bernaerts, Vangrunderbeek, et al. (2022). As shown in in **Figure 4**, the potential of pretreatments to steer (bio)chemical conversions still holds if followed by a pasteurization step. Moreover, refrigerated storage was not seen to exert a major influence on the relative differences between the differently pasteurized pretreated samples as observed in the biplots (**Figure 4**). VID, identity, chemical class and RI of the discriminant markers are shown in **Table 3**.

3.1.2.2 Interpretation of the identity and relative abundance of selected discriminant compounds in pretreated pasteurized Brussels sprouts

Bar plots as a summation of all compounds belonging to one chemical class of selected discriminant aldehydes and alcohols, nitriles, isothiocyanates and sulfurous compounds are represented in **Figure 5**.

As for the alcohols, volatiles related to the PUFAs-LOX-HPL pathway, namely **(Z)-3-hexen-1-ol**, **1-hexanol**, **(Z)-2-hexenal**, **(E)-2-hexenal**, **1-penten-3-ol** and **(Z)-2-penten-1-ol** are abundantly observed after mixing.

Their presence can be linked to the enzymatic (and/or non-enzymatic) conversion of PUFAs by LOX (and/or via autoxidation) generating hydroperoxides which could subsequently be further converted to (C6) aldehydes by HPL followed by conversion to alcohols by ADH. More specifically, the presence of (E)-2-hexenal and 3-hexen-1-ol, on the one hand and 1-hexanol, on the other hand can be addressed to the enzyme-catalyzed oxidation of α-linolenic acid and linoleic acid, respectively (Grebenteuch et al., 2021; Vincenti et al., 2019). Besides, thermally induced reactivities and autoxidation could have played a role in defining the final abundances (Cao et al., 2014; Engelberth & Engelberth, 2020; Grebenteuch et al., 2021; Vincenti et al., 2019). However, thermal degradation of PUFAs leading to these compounds is expected to have taken place to a limited extent since otherwise, the latter compounds would also probably have been arisen in the headspace after NoPT + Past, in which PUFAs were expected to still be more abundantly present before initiation of the pasteurization process.

The assumed occurrence of enzyme-substrate interactions in the mixed samples is also reflected by the presence of products related to the GSLs-MYR pathway. Nitriles, well-known enzymatic reaction products derived from GSLs, catalyzed by MYR, such as **benzyl nitrile**, **4,4-dimethyl-3-oxopentanenitrile** and **3-methyl butanenitrile** are more abundant in the mixed samples in which no subsequent incubation step was followed. This might be an indication of the conversion of these reaction products into other products through the incubation step during the Mix + Inc + Past treatment (**Figure 5**).

Besides enzymatic formation, nitriles are well-known compounds derived from the thermal degradation of GSLs, as reported in studies on broccoli sprouts, kohlrabi, white and red cabbage and broccoli (Hanschen et al., 2012, 2018; Kebede et al., 2013).. Thermal degradation of GSLs leading to the formation of nitriles might be another plausible explanation for the observed higher abundance of nitriles in the headspace of the Mix + Past sample compared to the amount of nitriles in the Mix + Inc + Past sample. After mixing without incubation, more GSLs could have still been present in the sample (as compared to the presence of GSLs the Mix + Inc sample), which are prone to be subjected to thermally induced degradation leading to nitriles.

Other well-known reaction products, implicated to be derived from the GSLs-MYR pathway, are in particular observed in the headspaces of the mixed samples, namely **1-isothiocyanato-3-methyl-butane**, **2-methylbutyl isothiocyanate**, **isobutyl isothiocyanate**, **allyl isothiocyanate** and **isothiocyanato cyclopropane**, again

addressed to the extensive tissue disruptive step enabling substrate-enzyme interaction. As has been stated in literature, isothiocyanates are known to be present in mildly heat-treated cut vegetables as mild heat-treatment causes the inactivation of the ESP (a cofactor which favors the formation of nitriles) while MYR is still active, leading to the favored formation of isothiocyanates (Bones & Rossiter, 2006; Hanschen et al., 2018). Similar as for the nitriles, the abundance of the latter compounds are seen to be significantly higher in the headspace of the mixed sample that did not undergo an additional incubation step at 40 °C. This might again be explained by the possible further conversion of the latter compounds during/after incubation into other compounds and/or the presence of those isothiocyanates due to thermal degradation of the present substrates (i.e., GSLs) (Deng et al., 2015). More specifically, it is expected that still more substrates prone to be (bio)chemically converted leading to isothiocyanates, are present in the mixed sample lacking an incubation step at the level of the pasteurization step.

Finally, sulfurous compounds such as **dimethyl trisulfide**, **dimethyl disulfide** and **methyl(methylthio)methyl disulfide** are as well prominently present in the mixed samples. Regarding these compounds, the incubation step does seem to exert an influence on their additional formation as observed in their higher abundance after Mix + Inc + Past in comparison to their presence after mixing and pasteurization without the added incubation step. Besides, also heat-induced reactivities could have presumably determined the final abundance in the different headspaces for which the physical state of the system (i.e., NoPT, mixed system) on which the heat step (i.e., pasteurization step) was applied could additionally have been an important aspect (Bones & Rossiter, 2006; Deng et al., 2015).

Notably, particular aldehydes as **hexanal** and **pentanal** and mainly particular nitriles, namely **2-butenenitrile** and **3-methyl-2-butenenitrile** are observed to a greater extent in headspace of the NoPT + Past sample. The presence of these compounds in this sample might be addressed to possible enzyme-substrate interactions as a result of a possible (minor) tissue wounding by/after the harvest and/or to thermally induced reactions leading to these compounds. Since the NoPT + Past sample for Brussels sprouts did not undergo a chopping process, thermally induced reactions seem to be more reasonable compared to the enzymatic conversions. Nonetheless, it is not excluded that enzymatic conversion to a certain extent could have occurred upon harvesting.

3.2 Influence of refrigerated storage on the volatile profile of pasteurized vegetable products

As speculated in the first part of this paper, the kind of pretreatment predominantly affected the volatile profile of pasteurized leek and Brussels sprouts compared to the effect of the refrigerated storage (*cfr*., section 3.1). However, particular (minor) changes in the volatile profile of those products upon refrigerated storage do still occur, on which the following section will focus. These changes could be important regarding specific flavor-active compounds which can exceed or go below their sensorial concentration threshold upon storage (data not collected), hence, impacting the consumers perception and consequently acceptance of cooled stored (pretreated) pasteurized vegetable products. This was not the aim in current study, but could be relevant to include in follow-up experiments.

3.2.1 Leek

3.2.1.1 Qualitative and quantitative classification of the volatile profiles

Differently pretreated pasteurized leek, stored in a refrigerator at 4 °C for 4 weeks were analyzed using an untargeted GC-MS fingerprinting approach. In the headspace of the stored NoPT + Past, Mix + Past and Mix + Inc + Past samples, a total of 144, 146 and 142 volatile compounds, respectively were detected. PLS regression was used as a modelling technique on the continuous Y data sets to describe the changes in volatile composition during storage for each of the samples. For the NoPT + Past sample, 4 LVs were used to build the model, explaining in total 98.13% of the total Y-variance. For the Mix + Past and Mix + Inc + Past samples, models were based on 3 LVs, explaining 97.33 and 98.50% of the total Y-variance, respectively. The amount of markers based on a VID threshold of 0.7, was for the NoPT + Past, the Mix + Past and the Mix + Inc + Past sample 3, 17 and 12, respectively, accounting for 2.0, 11.5 and 8.5% of all the detected volatiles. As seen in these relatively low percentages and as deducible from **Figure 6**, the abundance of most volatiles does not change significantly upon refrigerated storage, as observed in the positions of open circles (i.e., depicted in the center of the biplot). These minor changes in volatile profile during refrigerated storage are to be expected given that (i) enzyme-catalyzed conversions during storage are expected to be non-existing/limited due to the thermal inactivation of enzymes during the pasteurization step, and (ii) a selected storage temperature of 4 °C largely reduces the rate

of chemical reactions (assuming a $Q_{10\,°C} = 2$, a value quantifying the temperature sensitivity of many chemical reactions) (Wu et al., 2021).

Open circles positioned more to the right hand side of the biplot correspond to volatiles that are more abundant when stored for longer times, which is the opposite for volatiles depicted more on the left hand side of the biplot. VID, identity, chemical class and RI of markers can be found in **Table 4**. A major observation is that, except for one component, all markers have negative VID coefficients indicating these marker volatiles are being degraded during refrigerated storage.

3.2.1.2 Interpretation of the observed evolution of selected discriminant compounds throughout storage

Dipropyl disulfide, (Z)-2-hexenal, propanal and **2-ethyl-1-hexanol** were selected for discussion for which the presence can be related to PUFA conversion by LOX and ACSOs conversion by ALL, but could also be (partially) ascribed to thermal reactivities induced by pasteurization. Abundances of those selected compounds throughout storage are depicted in **Figure 7**. VIDs of dipropyl disulfide, (Z)-2-hexenal and propanal possess negative values in the mixed samples signifying their decrease upon storage, which can also be observed in the biplots (**Figure 6**). Since the pasteurization process ($P_{90\,°C}^{10\,°C}$= 10-11 min) allowed POD negative activity, it could be assured enzymatic conversions throughout storage were excluded as POD is considered to be the most heat-stable enzyme in vegetables (McLellan & Robinson, 1981). Therefore, the observed decrease can be addressed to the non-enzymatic breakdown of these compounds in those samples, induced by cold storage. 2-Ethyl-1-hexanol possesses a positive VID upon storage in the NoPT + Past sample, which can be related to storage-induced (non-enzymatic) formation. However, it must be kept in mind that these changes are expected to be relatively small as storage took place at a relatively low temperature.

3.2.2 Brussels sprouts

3.2.2.1 Qualitative and quantitative classification of the volatile profiles

A PLS regression model consisting of 3 LVs explaining 98.78% of the total Y-variance for continuous Y data sets (modelling the evolution upon storage) after volatile profile analyses of cooled stored NoPT + Past samples of Brussels sprouts, gave a detection of a total of 93 volatiles, of which 13 were referred to as markers (i.e., 14.0%). For the Mix + Past data set, 142 volatiles were detected, of which 12 were markers (i.e., 8.5%). Finally, after Mix + Inc + Past, 146 volatiles were detected over all cooled stored samples for which three were indicated

as discriminant (i.e., 2.1%). Based on the above (relatively low) amounts of markers in the samples and as can be derived from the biplots (**Figure 8**), similar as for leek (*cfr.,* 3.2.1), the major part of the volatiles did not change significantly upon storage, which verifies the results as discussed in **section 3.1.2.1** and could also be hypothesized based on the $Q_{10\,°C} = 2$ value (Wu et al., 2021). VID, identity, chemical class and RI of the markers are given in **Table 5**.

3.2.2.2 Interpretation of the observed evolution of selected discriminant compounds throughout storage

Notwithstanding the relatively minor change upon refrigerated storage, **2-butenenitrile, benzyl nitrile, allyl isothiocyanate, 2-hexenal** and **hexanal** were selected markers for discussion, first, by their discriminant behavior as concluded based on the VID criterion and, second, by their possible formation via both the GSLs-MYR pathway and the PUFAs-LOX-HPL pathway. On the one hand, an increase in the abundance of 2-butenenitrile and benzyl nitrile, possible reaction products of the conversion of GSLs by MYR, and hexanal, a possible a reaction product of the conversion of PUFAs catalyzed by LOX and HPL was observed. Those products might also be a result of thermal reactivities. On the other hand, a decrease in allyl isothiocyanate, also a product of the GSLs-MYR (thermal) pathway, was seen in the headspace of the stored NoPT + Past sample. These observations could be related to both storage induced formation and degradation from/into other compounds. The presence of 2-butenenitrile, benzyl nitrile, allyl isothiocyanate, 2-hexenal and hexanal in the NoPT + Past sample in the beginning of the storage can be related to prior minor tissue wounding during the harvest and/or postharvest. Only a decrease in compounds that show a discriminant behavior upon storage could be seen in the volatile headspace of the Mix (+ Inc) + Past samples, as also observed in the biplots (**Figure 8b and c**). 2-Butenenitrile, 2-hexenal and hexanal have probably been degraded into other compounds upon storage (**Figure 9**). Again, the presence of these compounds in the beginning of the storage period could be related to important quality-related pathways in Brussels sprouts.

582

583 **4. Conclusions**

584 In this study, the impact of various pretreatments that aimed to steer (bio)chemical conversions in leek and
585 Brussels sprouts and the consecutive changes upon refrigerated storage of the volatile profile of pasteurized leek
586 and Brussels sprouts products was investigated. Outcomes of this study clearly showed that varying processing
587 steps/conditions during pretreatment can be used to steer (bio)chemical conversions in leek and Brussels sprouts
588 and that the impact of refrigerated storage was seen to limitedly impact the volatile profile (relatively compared
589 to the impact of prior pretreatment). Presence of compounds in pretreated pasteurized Brussels sprouts and leek
590 could be related to several (combinations of) consecutive (bio)chemical reactions. For both vegetables, mixing
591 seemed to clearly provoke enzyme-substrate reactivities, ascribed to the extensive decompartmentalization.
592 Unexpectedly, an additional incubation step after mixing did not induce further enzymatic conversions, induced
593 by mixing. The latter observation can most possibly be ascribed to the probable complete conversion of ACSOs,
594 GSLs and/or PUFAs before the pasteurization was initiated.

595 The impact of pretreatment was more dominant compared to the impact of cooled storage, implying that this
596 step of the food processing chain is believed to be a main contributing factor determining the quality. However,
597 as refrigerated storage was also seen to exert a particular influence on the abundance of possible flavor-imparting
598 compounds in intact or disintegrated pasteurized Brussels sprouts and leek, the time of refrigerated storage
599 should also be taken into account when storing a pasteurized product in the context of preference and/or
600 acceptance of a product.

601 Since this work focused on the qualitative comparison of differently treated and stored products, it would be of
602 relevance in future work to determine the absolute quantity of relevant compounds and/or to implement *in vivo*
603 sensory tests to get detailed insight into the possible flavor-active properties and perception (acceptance and/or
604 preference (upon storage)) (by linking absolute quantities with sensory threshold of specified compounds found
605 in the literature) of the products and to investigate whether the differences based on the instrumental analysis in
606 volatile profiles and trends of changes in volatile profiles are also perceived by humans.

607

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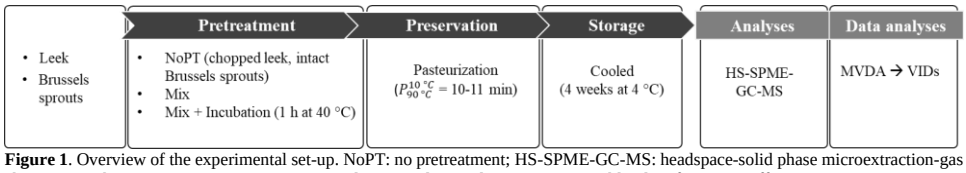

Figure 1. Overview of the experimental set-up. NoPT: no pretreatment; HS-SPME-GC-MS: headspace-solid phase microextraction-gas chromatography-mass spectrometry; MVDA: multivariate data analysis; VIDs: Variable identification coefficients.

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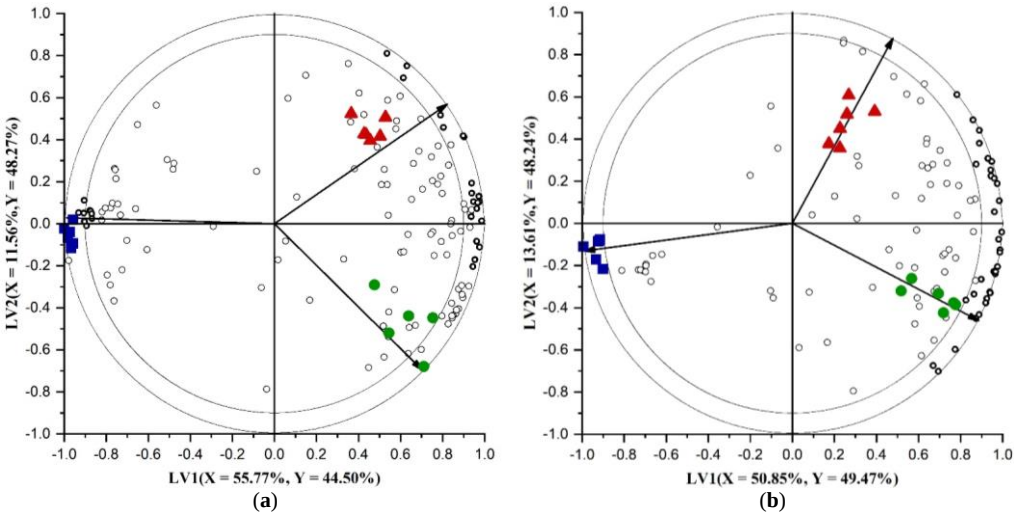
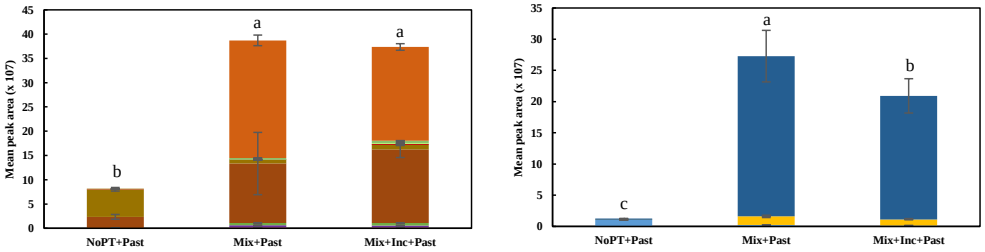


Figure 2. Partial Least Squares-Discriminant Analyses-based biplots of latent variable 2 (LV2) as a function of LV1 visualizing the effect of pretreatment (**a**) in the beginning and (**b**) at the last day of refrigerated storage of pasteurized leek products ((■) no pretreatment (NoPT) + pasteurization (Past), (●) Mix + Past, and (▲) Mix + incubation (Inc) + Past). The X- and Y-variance (%) explained by each LV are indicated in the respective axes. The vectors represent the correlation loadings for the estimated Y-variables. The inner and outer circles depict the correlation coefficient of 0.9 and 1.0, respectively. Volatile compounds are indicated as open circles (o) with the discriminant volatile compounds depicted in bold (**o**) (| Variable identification (VID) coefficient | ≥ 0.9).



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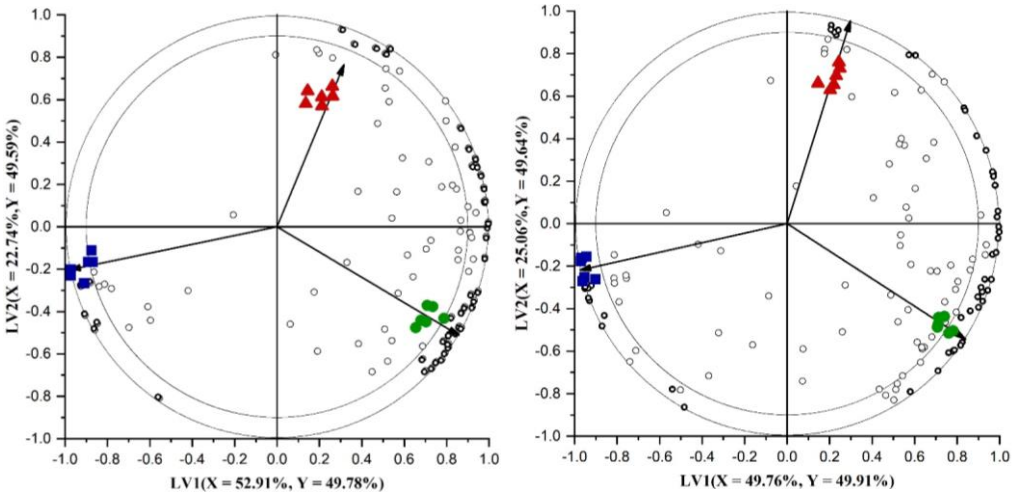
798 **Figure 3**. Specific compound plots of selected discriminant (**a**) aldehydes and (**b**) sulfurous compounds present in the headspace of
799 differently pretreated pasteurized leek in the beginning of the storage period. The most abundant discriminant compounds over all
800 samples are (■) hexanal, (■) (E)-2-hexenal, (■) pentanal, (■) dimethyl trisulfide, (■) methyl-2-propenyl trisulfide, and (■) (E)-1-(prop-
801 1-en-1-yl)-3-propyltrisulfane. Other identified discriminant alcohols and aldehydes comprised (E,E)-2,4-heptadienal, hexanal,
802 benzaldehyde, (E)-2-hexenal, 3-ethyl-4-nonenal, 2-methyl-2-pentenal, 2-methyl-pentanal and 2-methyl-2-butenal and other discriminant
803 sulfurous compounds were methyl-2-propenyl trisulfide, allyl n-propyl sulfide and methyl propyl disulfide. Statistically significant
804 differences between mean peak areas for each of the summated compounds per class are designated by different letters ($p < 0.05$, $n =$
805 6). NoPT: no pretreatment, Past: pasteurization; Inc: incubation.



(**a**) (**b**)

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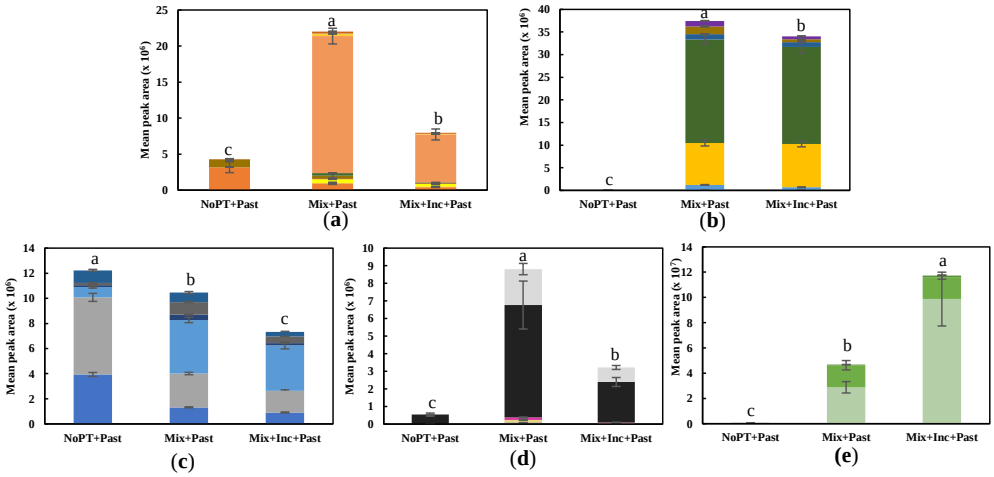
807 **Figure 4.** Partial Least Squares-Discriminant Analyses-based biplots of latent variable 2 (LV2) as a function of
808 LV1 visualizing the effect of pretreatment on (**a**) the first and (**b**) the last day of refrigerated storage of
809 pasteurized Brussels sprouts products ((■) no pretreatment (NoPT) + pasteurization (Past), (●) Mix + Past, and
810 (▲) Mix + incubation (Inc) + Past). The X- and Y-variance (%) explained by each LV are indicated in the
811 respective axes. The vectors represent the correlation loadings for the estimated Y-variables. The inner and outer
812 circles depict the correlation coefficient of 0.9 and 1.0, respectively. Volatile compounds are indicated as open
813 circles



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815 **Figure 5**. Specific compound plots of selected discriminant (**a**) aldehydes, (**b**) alcohols, (**c**) nitriles, (**d**) isothiocyanates and (**e**) sulfurous
816 compounds present in the headspace of differently pretreated pasteurized Brussels sprouts in the beginning of the storage period. The
817 most abundant discriminant compounds over all samples are (■) pentanal, (■) 2-hexenal, (■) (Z)-3-hexen-1-ol, (■) 1-hexanol, (■) 2-
818 butenenitrile, (■) 3-methyl-2-butenenitrile, (■) benzyl nitrile, (■) allyl isothiocyanate, (■) isothiocyanato cyclopropane, (■) dimethyl
819 trisulfide, and (■) dimethyl trisulfide. Names of other, less abundant discriminant compounds can be found in **Table 3**. Statistically
820 significant differences between mean peak areas for each of the summated compounds per class are designated by different letters ($p <$
821 0.05, $n = 6$). NoPT: no pretreatment; Past: pasteurization; Inc: incubation.

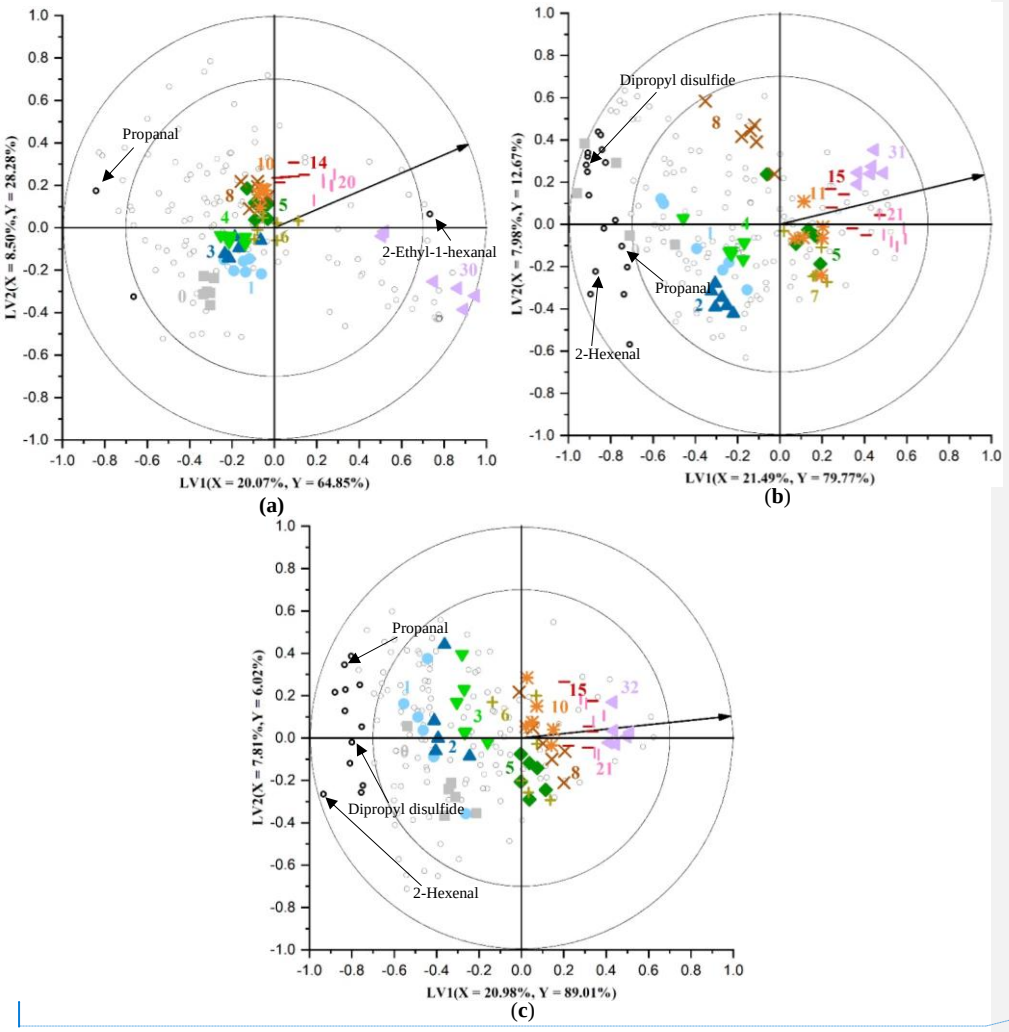

Figure 6. Partial Least Squares regression biplots of latent variable 2 (LV2) as a function of LV1 describing the effect of refrigerated storage on the volatile profile of differently pretreated leek (in storage time (days)). (**a**) no pretreatment (NoPT) + pasteurization (Past): (■) 0, (●) 1, (▲) 3, (▼) 4, (◆) 5, (┼) 6, (×) 8, (✳) 10, (–) 14, (|) 20, (◀) 30; (**b**) Mix + Past: (■) 0, (●) 1, (▲) 2, (▼) 4, (◆) 5, (┼) 7, (×) 8, (✳) 11, (–) 15, (|) 21, (◀) 31; (**c**) Mix + incubation (Inc) + Past: (■) 0, (●)1, (▲) 2, (▼) 3, (◆) 5, (┼) 6, (×) 8, (✳) 10, (–) 15, (|) 21, (◀) 32. The *X*- and *Y*-variance (%) explained by each LV are indicated in the respective axes. The vectors

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represent the correlation loadings for the estimated Y-variables. Volatile compounds are indicated as open circles (o) with the discriminant volatile compounds depicted in bold (**o**) (| Variable identification (VID)

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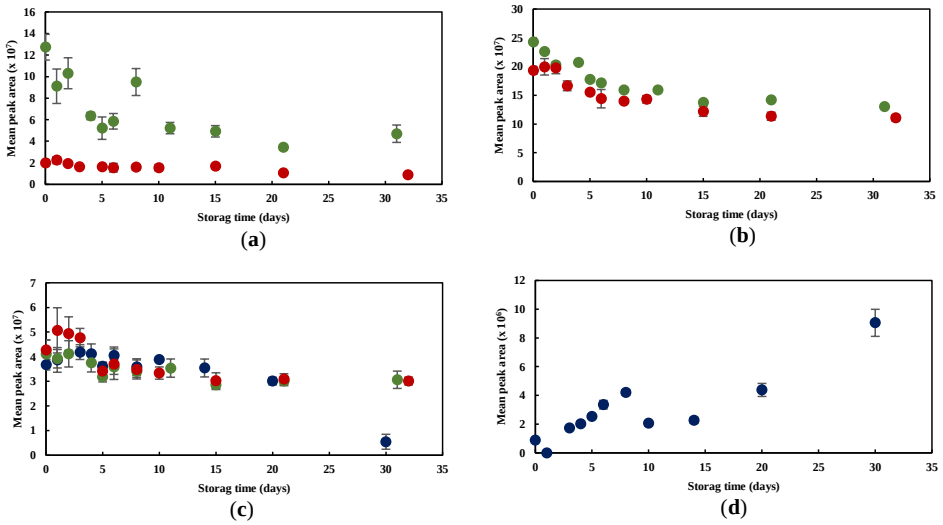
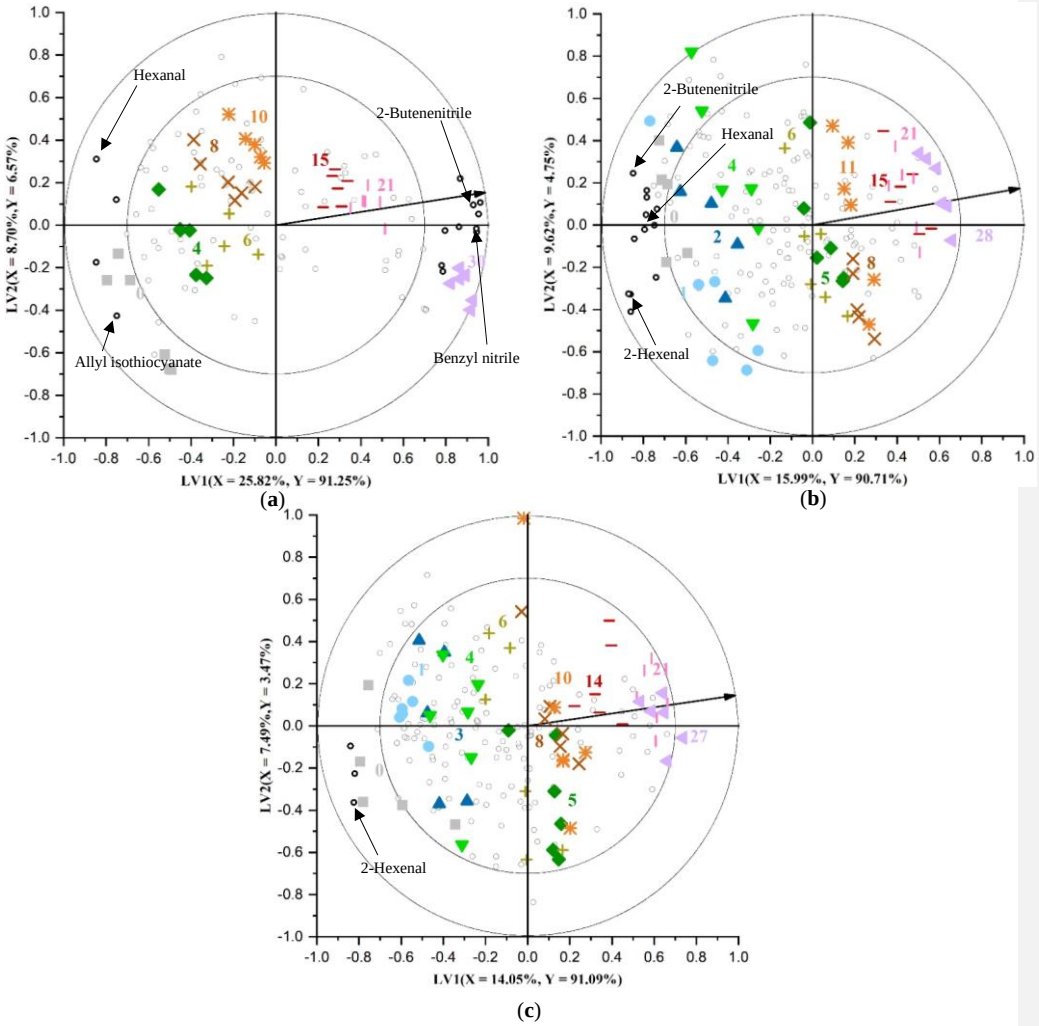


Figure 7. Evolution of discriminant compounds throughout storage for 4 weeks at 4 °C in the headspaces of differently pretreated pasteurized leek ((●) no pretreatment (NoPT) + pasteurization (Past), (●) Mix + Past, (●) Mix + incubation (Inc) + Past). (**a**) dipropyl disulfide, (**b**) 2-hexenal, (**c**) propanal, and (**d**) 2-ethyl-1-hexanol (n = 6).



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839 **Figure 8.** Partial Least Squares regression biplots describing the effect of refrigerated storage on the volatile profile of differently
840 pretreated pasteurized Brussels products (in storage time (days)). (**a**) no pretreatment (NoPT) + pasteurization (Past): (■) 0, (◆) 4, (┼)
841 6, (×) 8, (✳) 10, (–) 15, (|) 21, (◀) 30; (**b**) Mix + Past: (■) 0, (●) 1, (▲) 2, (▼) 4, (◆) 5, (┼) 6, (×) 8, (✳) 11, (–) 15, (|) 21, (◀)
842 28; (**c**) Mix + incubation (Inc) + Past: (■) 0, (●)1, (▲) 3, (▼) 4, (◆) 5, (┼) 6, (×) 8, (✳) 10, (–) 14, (|) 21, (◀) 27. The *X*- and *Y*-
843 variance (%) explained by each latent variable (LV) are indicated in the respective axes. The vectors represent the correlation loadings
844 for the estimated *Y*-variables. Volatile compounds are indicated as open circles (o) with the discriminant volatile compounds depicted
845 in bold (**o**) (|Variable identification (VID) coefficient|≥ 0.7). 2-Butenenitrile, benzyl nitrile, 2-hexenal, hexanal and allyl isothiocyanate
846 are indicated in the biplots of the treatments for which the respective compounds are discriminant. The inner and outer ellipse of the
847 biplot depict the correlation coefficient of 0.7 and 1.0, respectively.

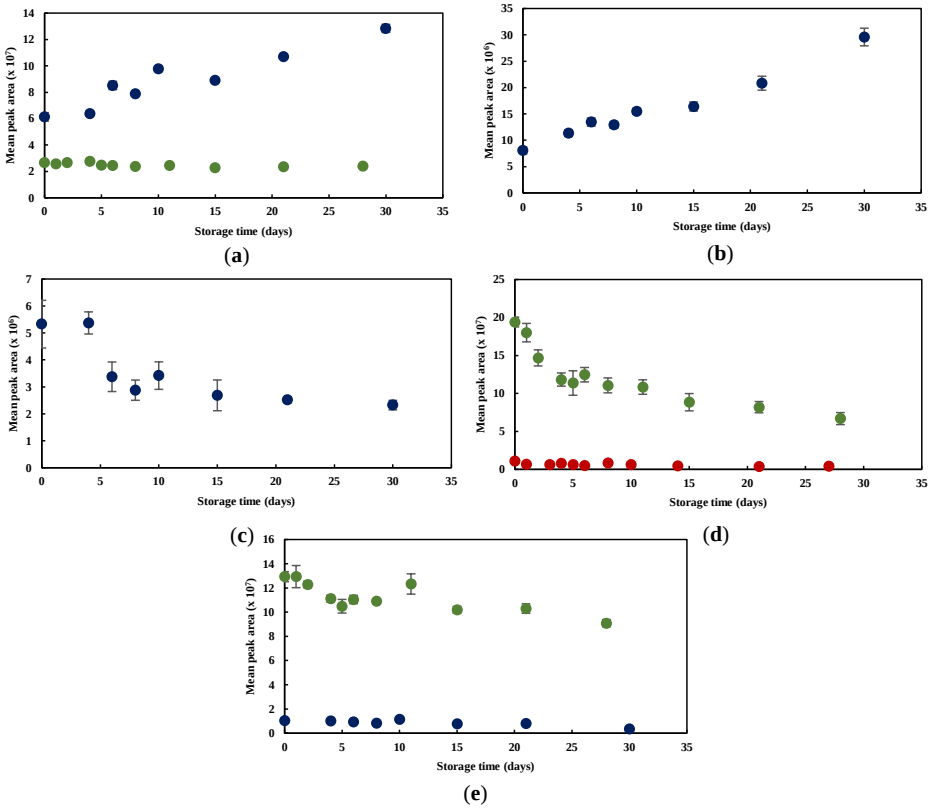

Figure 9. Evolution of discriminant compounds throughout storage for 4 weeks at 4 °C in the headspaces of differently pretreated pasteurized Brussels sprouts ((●) no pretreatment (NoPT) + pasteurization (Past), (●) Mix + Past, (●) Mix + incubation (Inc) + Past). (**a**) 2-butenenitrile, (**b**) benzyl nitrile, (**c**) allyl isothiocyanate, (**d**) 2-hexenal, and (**e**) hexanal (n = 6).

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Table 1. The profiles of the pasteurization cycle for each pretreated sample. NoPT: no pretreatment; Inc: incubation.

Pretreatment	Coming up time to 40 °C (min)	Incubation time at 40 °C (min)	Coming up time to 90 °C (min)	Holding time at 90 °C (min)	Cooling time (min)
NoPT	-	-	13	14 (Brussels sprouts)/ 16 (leek)	15
Mix	-	-	13	15	15
Mix + Inc	3	90	9	15	15

Table 2. Variable identification (VID) coefficient, identity, chemical class and Retention Index (RI) of markers ($|VID| \geq 0.9$) for differently pretreated and pasteurized leek in the beginning of the storage period.* NoPT: no pretreatment; Incl incubation.

NoPT + Past			
VID	**Identity**	**Chemical class**	**RI**
0.969	*Unidentified*	-	712
0.956	Pentanal	Aldehyde	985
0.954	*Unidentified*	-	1825
0.947	(E)-1-(Prop-1-en-1-yl)-3-propyltrisulfane	Sulfurous compound	1803
0.944	2-Methyl-2-ethoxy propane [a]	Alkane	750
0.917	Propyl mercaptan	Sulfurous compound	837
0.914	*Unidentified*	-	733
0.911	2,3,5-Trimethyl hexane [a]	Alkane	1000
0.906	Bis (n-propylthio) methane [a]	Sulfurous compound	1512
-0.903	Butyl ester acetic acid	Ester	1082
-0.904	1-Chloro pentane	Haloalkane	944
-0.905	Hexanal	Aldehyde	1094
-0.907	Trans-β-Ionone [a]	Ketone	1962
-0.911	Methyl-2-propenyl trisulfide	Sulfurous compound	1611
-0.919	Dimethyl trisulfide	Sulfurous compound	1396
-0.931	2-Methyl-2-pentenal	Aldehyde	1166
-0.932	*Unidentified*	-	1469
-0.941	(E,E)-2,4-Heptadienal	Aldehyde	1510
-0.943	3-Ethyl-2,5-dimethyl-1,3-hexadiene [a]	Alkene	1527
-0.943	2-Ethyl-trans-2-butenal	Aldehyde	1169
-0.945	(Z)-2-Hexenal	Aldehyde	1228
-0.954	(E)-2-Hexenal	Aldehyde	1211

Mix + Inc + Past			
VID	**Identity**	**Chemical class**	**RI**
0.977	2-Methyl-3-methylene cyclopentanecarboxaldehyde [a]	Cycloaldehyde	1477
0.976	2-Methyl-2-butenal [a]	Aldehyde	1107
0.947	3-Ethyl-4-nonenal [a]	Aldehyde	1662
0.944	1-Methyl-methyl ester hydrazinecarbodithioic acid [a]	Sulfurous compound/N-compound	1749
0.922	1-(Methylthio) heptane [a]	Sulfurous compound	1634
0.902	Benzaldehyde	Aldehyde	1543
0.900	2-Methyl pentanal [a]	Aldehyde	1011

*Components, identified using the spectral library of NIST, that are not matching with the RI found in literature, are indicated as '*unidentified'*. Components for which the RIs are not found in literature are indicated as '*tentatively identified*' ([a]). The components 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 of that compound in that specific class.

Table 3. Variable identification (VID) coefficient, identity, chemical class and Retention Index (RI) of markers ($|VID| \geq 0.9$) for differently pretreated and pasteurized Brussels sprouts in the beginning of the storage period.* NoPT: no pretreatment; Past: pasteurization; Inc: incubation.

NoPT + Past			
VID	**Identity**	**Chemical class**	**RI**
0.971	3-Methyl-2-butenenitrile [a]	Nitrile	1284
0.962	Methanethiol	Sulfurous compound	744
0.952	*Unidentified*	-	2012
0.940	2-Butenenitrile	Nitrile	1192
0.922	Pentanal	Aldehyde	985
0.917	α,α-dimethyl cycloheptanemethanol[a]	Cycloalcohol	903
-0.931	(Z)-2-Penten-1-ol	Alcohol	1330
-0.941	2-Ethyl-2-pentenal [a]	Aldehyde	1260
-0.957	4-Ethyl benzaldehyde	Aldehyde	1732
-0.959	Nitro cyclopentane [a]	N-compound	1496
-0.960	Dimethyl disulfide	Sulfurous compound	1087
-0.971	2-Ethyl thiophene	Sulfurous compound	1185
-0.980	Methyl ester thiocyanic acid	Ester	1286
-0.981	Cyano-3,4-epithiobutane[a]	Sulfurous compound	1978
-0.981	1-Hexanol	Alcohol	1359
-0.982	2-Methyl-3-methylene cyclopentanecarboxaldehyde [a]	Cycloaldehyde	1427
-0.986	Benzyl nitrile	Nitrile	1959
-0.990	(Z)-3-Hexen-1-ol	Alcohol	1392
-0.993	(E)-3-Hexen-1-ol	Alcohol	1370

Mix + Past			
VID	**Identity**	**Chemical class**	**RI**
0.990	*Unidentified*	-	1634
0.989	Hexanal	Aldehyde	1094
0.985	Butanal	Aldehyde	881
0.986	3-Methyl butanenitrile	Nitrile	1138
0.985	(Z)-2-Hexenal	Aldehyde	1228
0.982	(E)-2-Hexenal	Aldehyde	1211
0.979	Ethyl acetate	Ester	894
0.977	1-Penten-3-ol	Alcohol	1166
0.976	2,4-Decadienal	Aldehyde	1830
0.972	2-Methylbutyl isothiocyanate	Isothiocyanate	1436
0.970	4,4-Dimethyl-3-oxopentanenitrile [a]	Nitrile	1244
0.969	1-Isothiocyanato-3-methyl butane	Isothiocyanate/alkane	1444
0.957	Isobutyl isothiocyanate[a]	Isothiocyanate	1330
0.954	Allyl isothiocyanate	Isothiocyanate	1374
0.954	Isothiocyanato cyclopropane [a]	Isothiocyanate/cycloalkane	1862
0.950	3-Ethyl-1,5-octadiene	Alkene	1013
0.950	Butyl ester acetic acid	Ester	1082
0.948	*Unidentified*	-	719
0.925	1,2,3-Trimethyl cyclohexane [a]	Cycloalkane	1344
0.916	(Z)-2-Penten-1-ol	Alcohol	1327
0.910	(E,E)-2,4-Heptadienal	Aldehyde	1506

Mix + Inc + Past			
VID	**Identity**	**Chemical class**	**RI**

0.979	Toluene	Aromatic hydrocarbon	1049
0.966	Methyl (methylthio)methyl disulfide	Sulfurous compound	1686
0.947	Dimethyl trisulfide	Sulfurous compound	1397
0.936	3,5,5-Trimethyl-3-cyclohexen-1-one	Ketone	1421
0.933	o-Xylene	Aromatic hydrocarbon	1195
-0.942	Isoamyl cyanide	Nitrile	1255

*Components, identified using the spectral library of NIST, that are not matching with the RI found in literature, are indicated as '*unidentified'*. Components, for which the RIs are not found in literature are indicated as '*tentatively identified*' ([a]). The components 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 of that compound in that specific class.

Table 4. Variable identification (VID) coefficient, identity, chemical class and Retention Index (RI) of markers ($|VID| \geq 0.7$) throughout storage for 4 weeks at 4 °C for differently pretreated and pasteurized leek.* NoPT: no pretreatment; Past: pasteurization; Inc: incubation.

NoPT + Past			
VID	**Identity**	**Chemical class**	**RI**
0.701	2-Ethyl-1-hexanol	Alcohol	1496
-0.724	Propanal	Aldehyde	1444
-0.731	Isopropyl isobutyl disulfide [a]	Sulfurous compound	2001

Mix + Past			
VID	**Identity**	**Chemical class**	**RI**
-0.713	Methyl propyl disulfide	Sulfurous compound	1243
-0.724	2,4-Dimethyl thiophene	Sulfurous compound	1202
-0.725	Propanal	Aldehyde	800
-0.725	Methyl-2-propenyl disulfide	Sulfurous compound	1293
-0.733	1-Allyl-2-isopropyldisulfane [a]	Sulfurous compound	1444
-0.744	2-Ethyl furan	Furanic compund	957
-0.746	*Unidentified*	-	1814
-0.772	1-Chloro pentane	Haloalkane	944
-0.797	*Unidentified*	-	1825
-0.802	Pentanal	Aldehyde	985
-0.804	(E)-1-(Prop-1-en-1-yl)-2-propyldisulfane	Sulfurous compound	1451
-0.811	3,4-Dimethyl thiophene	Sulfurous compound	1265
-0.812	4-(2-Hydroxyphenyliminomethyl) benzene-1,3-diol [a]	Alcohol	1427
-0.829	(Z)-1-(Prop-1-en-1-yl)-3-propyltrisulfane	Sulfurous compound	1803
-0.830	Dipropyl disulfide	Sulfurous compound	1389
-0.898	(E)-2-Hexenal	Aldehyde	1211
-0.958	(Z)-2-Hexenal	Aldehyde	1228

Mix + Inc + Past			
VID	**Identity**	**Chemical class**	**RI**
-0.729	Propyl mercaptan	Sulfurous compound	837
-0.731	2-Methyl-2-pentenal	Aldehyde	1166
-0.734	*Unidentified*	-	1825
-0.735	Methyl 2-propenyl disulfide	Sulfurous compound	1293
-0.761	Dipropyl disulfide	Sulfurous compound	1389
-0.771	*Unidentified*	-	1265
-0.772	*Unidentified*	-	1719
-0.799	Propanal	Aldehyde	800
-0.810	(E)-2-Hexenal	Aldehyde	1211
-0.831	1-Chloro pentane	Haloalkane	944
-0.855	Pentanal	Aldehyde	985
-0.953	(Z)-2-Hexenal	Aldehyde	1228

*Components, identified using the spectral library of NIST, that are not matching with the RI found in literature, are indicated as '*unidentified'*. Components, for which the RIs are not found in literature are indicated as '*tentatively identified*' ([a]). The components are listed in decreasing order of VID. A positive VID of a compound conveys the presence of a higher

concentration upon increased storage time whereas a negative VID denotes a lower concentration of that compound upon increase storage time.

Table 5. Variable identification (VID) coefficient, identity, chemical class and Retention Index (RI) of markers ($|VID| \geq 0.7$) throughout storage for 4 weeks at 4 °C for differently pretreated and pasteurized Brussels sprouts.* NoPT: no pretreatment; Past: pasteurization; Inc: incubation.

NoPT + Past			
VID	**Identity**	**Chemical class**	**RI**
0.972	3-Methyl butanenitrile [a]	Nitrile	1138
0.955	4,4-Dimethyl-3-oxopentanenitrile [a]	Nitrile	1244
0.941	2-Butenenitrile	Nitrile	1192
0.938	Benzyl nitrile	Nitrile	1959
0.937	4-(Methylthio)-butanenitrile [a]	Nitrile/sulfurous compound	1814
0.895	*Unidentified*	-	1284
0.865	3-Methyl-2-butenenitrile [a]	Nitrile	1284
0.799	Hexanenitrile	Nitrile	1309
0.754	Heptane	Alkane	748
-0.744	*Unidentified*	-	712
-0.777	Hexanal	Aldehyde	1094
-0.808	Allyl isothiocyanate	Isothiocyanate	1380
-0.868	Methanethiol	Sulfurous compound	744

Mix + Past			
VID	**Identity**	**Chemical class**	**RI**
-0.711	3,5,5-Trimethyl-3-cyclohexen-1-one	Ketone	1421
-0.716	1-Penten-3-ol	Alcohol	1166
-0.720	Thiocyanic acid, methyl ester	Ester	1286
-0.720	3-Methyl butanenitrile	Nitrile	1138
-0.772	*Unidentified*	-	1634
-0.773	2-Butenenitrile	Nitrile	1191
-0.778	Hexanal	Aldehyde	1094
-0.783	2,4-Decadienal	Aldehyde	1830
-0.822	Butanal	Aldehyde	881
-0.907	(E)-2-Hexenal	Aldehyde	1211
-0.916	(E,E)-2,4-Heptadienal	Aldehyde	1506
-0.916	2-Hexenal	Aldehyde	1228

Mix + Inc + Past			
VID	**Identity**	**Chemical class**	**RI**
-0.854	3,5,5-Trimethyl-3-cyclohexen-1-one	Cycloketone	1421
-0.860	2-Hexenal	Aldehyde	1228
-0.862	Nitro cyclopentane [a]	Cycoalkane/N-compound	1496

*Components, identified using the spectral library of NIST, that are not matching with the RI found in literature, are indicated as '*unidentified'*. Components, for which the RIs are not found in literature are indicated as '*tentatively identified*' ([a]). The components are listed in decreasing order of VID. A positive VID of a compound conveys the presence of a higher concentration upon increased storage time whereas a negative VID denotes a lower concentration of that compound upon increase storage time.