Formation of Aroma Compounds and Lipoxygenase (EC 1.13.11.12) Activity in Unblanched Leek (*Allium ampeloprasum* Var. *Bulga*) Slices during Long-Term Frozen Storage

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The content of aroma compounds (dynamic headspace) and catalytic activity of lipoxygenase (LOX) (EC. 1.13.11.12) were analyzed in 15 mm unblanched leek slices seven times during 12 months of frozen storage. The aroma profile changed from consisting of almost only sulfur compounds such as dipropyl disulfide [concentration in fresh leek (FL) = 0.197 mg/L, concentration after 12 months of frozen storage (12M) = 0.0409 mg/L] and propyl (*E*)-propenyl disulfide (FL = 0.0437 mg/L, 12M = 0.00452 mg/L) in the fresh leeks to being dominated by numerous saturated and unsaturated aldehydes, such as hexanal (FL = 1.53 mg/L, 12M = 3.63 mg/L), (*E,E*)-2,4-nonadienal (FL = 0.000 mg/L, 12M = 0.0647 mg/L), and (*E,E*)-2,4-decadienal (FL = 0.129 mg/L, 12M = 0.594 mg/L) at the end of the storage period. The catalytic activity of LOX diminished throughout frozen storage, but ∼25% of the original activity was present after 12 months of storage.

INTRODUCTION

Commercially produced frozen leek slices are often stored for 1 year or longer, which makes it relevant to investigate the impact of this storage on the development of aroma compounds in the leek slices. Manufacturing of frozen vegetables normally includes a blanching step to destroy the catalytic activity of enzymes and prevent off-flavor formation. Leeks, however, are often processed without blanching, mainly because this has an undesirable effect on the texture. Consequently, enzymes are still present during frozen storage.

The aroma of freshly cut leek and other *Allium* species is dominated by numerous sulfur-containing volatile compounds originating from the decomposition of the odorless nonvolatile precursors *S*-alk(en)ylcysteine sulfoxides by the action of alliinase (EC 4.4.1.4) (*1*, *2*), as shown in **Figure 1**, Scheme 1. Because of compartmentation of alliinase in the vacuole and the cysteine sulfoxides in the cytoplasm (*2*, *3*) volatiles are not produced until cell rupture, for example, by cutting into slices. The products are pyruvate, ammonia, and various sulfenic acids depending upon which radical is present; the ones of common occurrence (*1*) are listed in **Figure 1**. These sulfenic acids are highly reactive (*4*) and will quickly combine to form thiosul-

finates (**Figure 1**, Scheme 2). Thiosulfinates are responsible for the odor of freshly cut leeks (*4*, *5*), but as they are relatively unstable (*1*, *6*), they will rearrange to form polysulfides and thiosulfonates (**Figure 1**, Scheme 3). Thiosulfonates are expelling sulfur dioxide to yield the corresponding monosulfide, and the final products of the reaction will end up being a combination of mono- and polysulfides with all of the possible combinations of the radicals listed in **Figure 1**. If the radical on the cysteine sulfoxide is (*E*)-1-propenyl-, thiopropanal-*S*-oxide (the lachrymatory factor) is formed almost exclusively (*7*) (**Figure 1**, Scheme 4). This compound is also unstable and will lead to the formation of propanal and 2-methyl-2-pentenal (*6*).

Another pathway of producing aroma compounds is initiated by the lipoxygenase (EC 1.13.11.12) (LOX)-catalyzed oxidation of polyunsaturated fatty acids with a *cis,cis*-pentadiene moiety. This leads to the formation of mainly aldehydes and alcohols, which could contribute to the aroma of fresh leeks but will probably appear as off-flavors when present in larger amounts (*8*, *9*). This is not very pronounced in the fresh leek because of the pungency of thiosulfinates and thiopropanal-*S*-oxide (*6*), although (*E*)-2-hexenal is reported to be a predominant peak in leek aroma (*4*). However, when leek slices are stored frozen for a longer period, the production of aldehydes will have an influence on the aroma profile as well, because of the diminishing content of sulfur compounds (*9*). The aroma compounds actually produced depend on in which position LOX acts on

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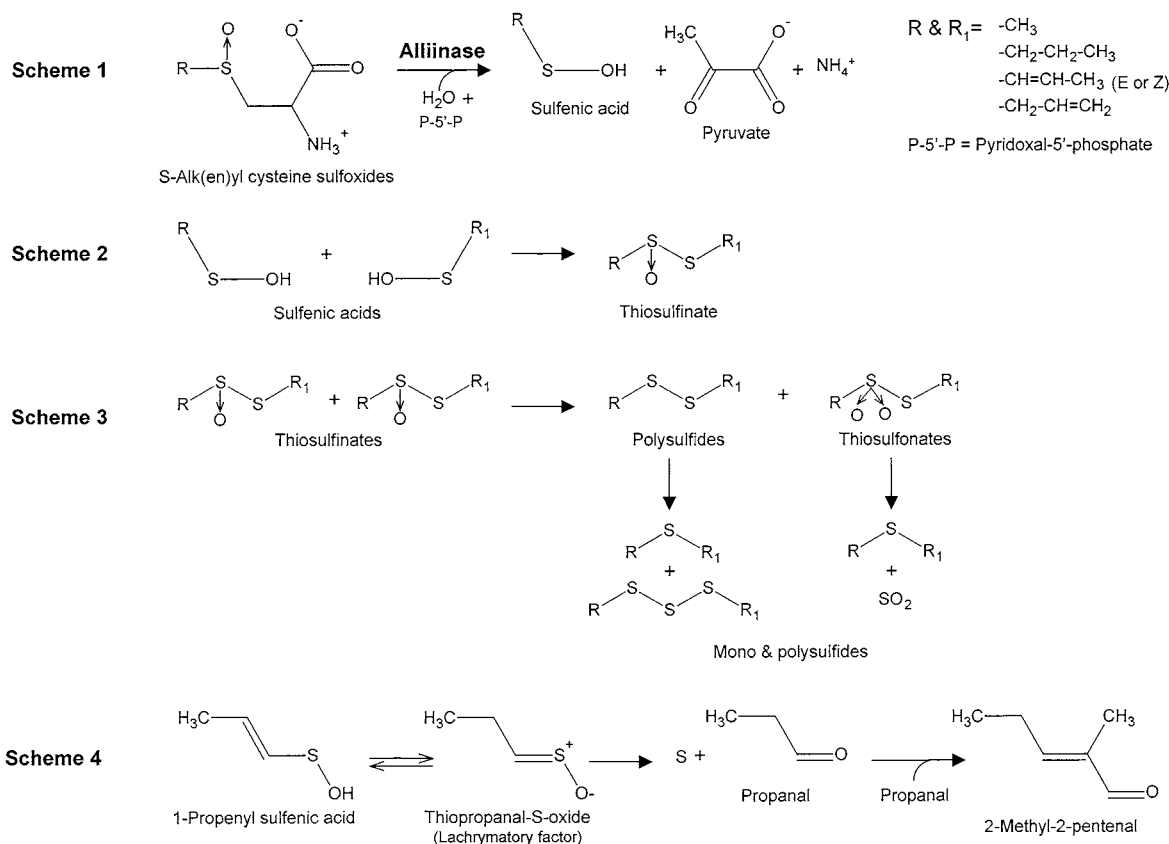*J. Agric. Food Chem.*, Vol. 51, No. 7, 2003 **1971**

Figure 1. Formation of sulfur-containing aroma compounds in leek and other *Allium* species.

the fatty acid and on the catalytic activities of hydroperoxide lyase and alcohol dehydrogenase. These compounds derived from fatty acids can also be generated by autoxidation during the storage period (*10*), and both pathways will result in accumulation of the products in the tissue.

Frozen leek slices are a commercial product in Europe, but hardly any investigations have been carried out on the changes in the aroma profile during freezing and frozen storage. The objective of the present study was to investigate the development of aroma compounds in leek slices during a long-term frozen storage. Furthermore, an examination of the catalytic activity of lipoxygenase in leek was included to correlate this activity with the development of aroma compounds originating from LOX-catalyzed oxidation of fatty acids.

MATERIALS AND METHODS

Plant Material. Leeks (*Allium ampeloprasum* var. *Bulga*) were harvested fully matured at Funen, Denmark, in October 1999 and immediately after used for the experiment.

Chemicals. All chemicals were of analytical grade and were bought commercially from Sigma-Aldrich.

Sample Preparation. The white part of the leek stem was cut into 15 mm slices and frozen immediately after in a blast freezer at −20 °C for 10 min, so that the center temperature reaches −20 °C. The slices were packed in airtight glass jars with atmospheric air as headspace and kept at −20 °C in the dark until analysis.

Experimental Design. Duplicates of samples were analyzed at harvest time and subsequently after 1, 2, 4, 6, 9, and 12 months of storage. Each sample was analyzed for catalytic activity of lipoxygenase and content of aroma compounds in three replicates.

Lipoxygenase Assay. Enzyme extract was made by mixing 50 g of frozen leek slices with 50 mL of phosphate buffer (0.2 M, pH 6.0) for 45 s in a Waring commercial blender. The slurry was kept on ice with agitation for 30 min (100 rpm), afterward filtered through a paper filter, and kept on ice until time of analysis. LOX was assayed by measuring

consumption of initial dioxygen by a Clark electrode (Digital Oxygen System model 10, Rank Brothers Ltd., Cambridge, U.K.) situated in a thermostatic cell (30 °C) with a magnetic stirrer using linoleic acid as the other substrate. Calibration was done at 30 °C by air-saturated phosphate buffer (0.2 M, pH 6.0) (21% dissolved oxygen) followed by the addition of sodium dithionite (0% dissolved oxygen). To determine lipoxygenase activity, 3.00 mL of air-saturated, tempered phosphate buffer was added to the thermostatic cell. After 30 s, 0.40 mL of enzyme extract was added, after which the lid was closed. At 80 s the reaction was initiated by adding 0.20 mL of sodium linoleate solution (10 mM) using a syringe. Dioxygen was monitored over 20 min by measuring the oxygen content every second.

Calculation of Activity. LOX activity was calculated as katals per kilogram of protein under the assumption that air-saturated phosphate buffer (0.2 M, pH 6) at 30 °C with a salt strength of 12.40 g/L contains 7.11 mg of O_2/L (*11*). To determine the activity, the oxygen level (millimoles of O_2) was plotted against time, and the steepest slope (millimoles of O_2 per second) on the curve was found using continuous linear regression over 20 s at a time. Katals per kilogram was calculated as moles of O_2 per second per kilogram of protein. Time elapsed from adding substrate to occurrence of the steepest slope was registered as the length of the lag phase (seconds).

Determination of Protein Content. Protein content in the enzyme extraction was determined by Coomassie brilliant blue according to the method described in US/EG Bulletin 1069 from Bio-Rad Life Science Group, Hercules, CA, using lyophilized bovine serum albumin as the standard protein.

Dynamic Headspace Analysis. Aroma compounds were isolated by dynamic headspace with nitrogen (purity = 99.8%) as purge gas. Frozen leek slices (100 g) were crushed with 150 mL of tap water and 2 mL of internal standard (50 ppm of 4-methyl-1-pentanol) for 90 s in a Waring commercial blender. After the mixture had been transferred to a 1 L glass flask by adding another 150 mL of tap water, it was left for 10 min at 30 °C with agitation (200 rpm) to equilibrate the temperature before purging. Aroma compounds were trapped on 100 mg of Tenax GR (mesh size = 60/80, Buchem bv, Apeldoorn, The Netherlands) for 60 min with a nitrogen flow of 200 mL/min.

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Desorption of aroma compounds was done thermally by a short-path thermal desorber (model TD-4, Scientific Instrument Services Inc., Ringoes, NJ). Desorption temperature was 250 °C for 3 min with a helium flow of 11 mL/min. Separation was performed by a GC-MS (HP G1800 A GCD system) with the following conditions: column, DB-Wax from J&W Scientific (30 m × 0.25 mm i.d. × 0.25 μm film thickness); carrier gas, helium; flow, 1 mL/min (constant); split ratio, 1:10; start pressure, 53 kPa; oven program, 40 °C for 10 min, 6 °C/min to 240 °C, constant at 240 °C for 30 min. The mass selective detector was operated in the electron ionization mode, and the *m*/*z* (mass/charge) ratio ranged between 20 and 450. Identification was done by probability-based matching with mass spectra in the G1035A Wiley library (Hewlett-Packard). Specification of the similarity between mass spectra of the unknown compound and the reference library compound is given as the quality. Values of 90 and above are reliable identifications. Identity was confirmed by checking with mass spectra and retention indices obtained in the laboratory from reference compounds for the following compounds: dimethyl disulfide, methyl propyl disulfide, dipropyl disulfide, dimethyl trisulfide, pentanal, hexanal, heptanal, octanal, nonanal, (*E*)-2-butenal, (*E*)-2-pentenal, (*E*)-2-hexenal, (*E*)-2-heptenal, (*E*)-2-octenal, (*E*)-2-nonenal, (*E*)-2-decenal, 2-methyl-(*E*)-2-butenal, 2-methyl-(*E*)-2-pentenal, (*E,Z*)-2,4-heptadienal, (*E,E*)-2,4-heptadienal, (*E,Z*)-2,4-nonadienal, (*E,E*)-2,4-nonadienal, (*E,Z*)-2,4-decadienal, (*E,E*)-2,4-decadienal, 3-octanone, β-ionone, 1-pentanol, 1-hexanol, 1-octen-3-ol, and 2-pentylfuran.

Quantification. The reference compounds dimethyl disulfide, methyl propyl disulfide, dipropyl disulfide, dimethyl trisulfide, pentanal, hexanal, heptanal, octanal, nonanal, (*E*)-2-butenal, (*E*)-2-pentenal, (*E*)-2-hexenal, (*E*)-2-heptenal, (*E*)-2-octenal, (*E*)-2-nonenal, 2-methyl-(*E*)-2-butenal, 2-methyl-(*E*)-2-pentenal, (*E,Z*)-2,4-heptadienal, (*E,E*)-2,4-heptadienal, (*E,Z*)-2,4-nonadienal, (*E,E*)-2,4-nonadienal, (*E,Z*)-2,4-decadienal, (*E,E*)-2,4-decadienal, 3-octanone, 1-pentanol, 1-hexanol, and 1-octen-3-ol were each dissolved in 400 mL of water and 1.3% sugar added, which equals the sugar content of leeks. Dynamic headspace analysis was performed on the solutions with the same flow, time, and temperature conditions as applied to the leek samples. Each reference compound was analyzed in triplicate in three concentrations. The obtained peak areas were used to calculate the concentration of the compounds in the leeks.

The retention time indices (RTI) of the volatile compounds were calculated with a mixture of hydrocarbons (C₉−C₂₆) as references according to the method of ref *12*.

Statistical Analysis. Analysis of variance was carried out on each of the aroma compounds using the one-way ANOVA procedure in the Analyst part of SAS, version 8.2, SAS Institute Inc., Cary, NC. The effect of the storage period was tested. Sample means were compared by Duncan's multiple-range test. A significance level of 5% was applied.

RESULTS AND DISCUSSION

Table 1 displays all of the aroma compounds identified in fresh leek and in leek slices stored frozen for 12 months. The table shows that the composition of aroma compounds changes from consisting of almost only sulfur compounds to being dominated by aldehydes, which are considered to be off-flavors in larger amounts (*8*, *9*). A total of 14 sulfur compounds were detected, which all more or less show the same pattern. Almost all of them decrease effectively during storage (**Figure 2** displays the development of selected compounds and **Table 2** displays the statistical differences), although some, for example, propyl (*E*)-propenyl trisulfide and dipropyl disulfide, increase during the first 2 months, which is in agreement with the study of ref *9*. 2-Methyl-2-pentenal, which is one of the breakdown products of thiopropanal-*S*-oxide (the lachrymatory factor) (*4*), shows the same development. Others, for example, methyl 2-propenyl disulfide and dimethyl disulfide, are not present at all after 4−6 months of storage (data not shown), whereas
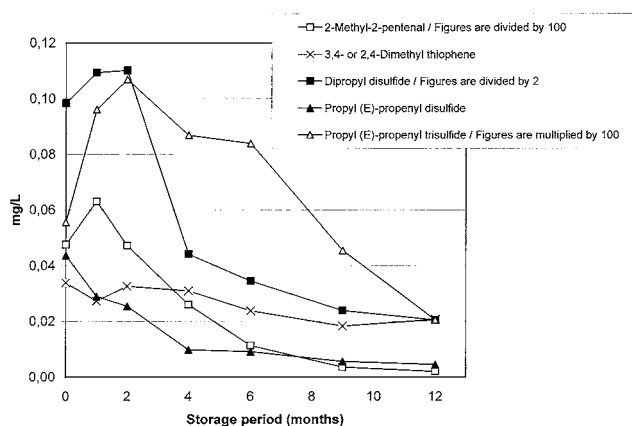

Figure 2. Development of selected sulfur compounds and 2-methyl-2-pentenal in leek slices during frozen storage. See **Table 2** for specification of statistical differences.

dimethylthiophene shows a very limited decrease throughout storage. When the concentrations of, for instance, dipropyl disulfide [fresh leek (FL) = 0.197 mg/L; leek stored frozen for 12 months (12M) = 0.0409 mg/L] and propyl (*E*)-propenyl disulfide (FL = 0.0437 mg/L, 12M = 0.00452 mg/L) in the fresh leek and after 12 months of frozen storage are compared with the threshold value [dipropyl disulfide = 0.0032 ppm and propyl (*E*)-propenyl disulfide = 0.0022 ppm (*13*)], the decrease is very likely to have an influence on the aroma profile of the frozen leek slices.

The production of these sulfur compounds is initiated by the action of alliinase (EC 4.4.1.4) upon *S*-(+)-alk(en)yl-L-cysteine sulfoxide, when cells are crushed. This means that the possibility of producing more sulfur compounds during the headspace analysis is present, when the leek slices are blended in water. This implies that alliinase is able to maintain catalytic activity after frozen storage, which very much depends on the treatment prior to the headspace analysis according to ref *3*. They found that activity was lost after slow freezing or thawing. This is not the case in this work, because the leek slices were quickly frozen in a blast freezer and blended frozen prior to the headspace analysis. Despite this, the present results indicate that either alliinase is not able to retain activity after a frozen storage or the substrate is not present in the right structure any more, because total content of sulfur compounds decreases from 0.544 to 0.110 mg/L.

No thiosulfinates were detected, although they most likely are present in the freshly cut leek slices (*4*). Considering our headspace and GC technique, this was expected, as the thiosulfinates are unstable. They will rearrange and dissociate both over time and by thermal exposure (*1*, *4*, *14−16*), and for that reason they will convert into mono- and polysulfides during the dynamic headspace collection and the GC-analysis. Thiosulfinates formed during processing and storage of the leeks will probably also decompose and will therefore not be present in the tissue at time of analysis.

Concentrations of saturated aldehydes (**Figure 3**) showed a substantial increase over the 12 month storage. Hexanal is the only one present in the fresh leek, whereas they all develop during storage. When the concentrations of the aroma compounds are compared with the odor thresholds [0.022 ppm for pentanal and 0.0025 ppm for hexanal (*17*)], there is no doubt that the storage period has an influence on the aroma profile. Hexanal is present at ∼1700 times the odor threshold after 12 months, whereas the amount of pentanal at that time is ∼20

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Table 1. Aroma Compounds Found in Fresh Leek and in Leeks Frozen for 12 Months

compound	quality[a]	RTI[b]	fresh leek, mg/L ± SD[c]	12 month storage, mg/L ± SD[c]	compound previously reported by[d]
sulfur compounds					
methyl pentyl sulfide[t]	96	1140	0.00 ± 0	0.000757 ± 0.00029	
dimethyl disulfide	97	1086	0.0232 ± 0.0062	0.00 ± 0	6,* 21, 22, 23, 24, 25, 26, 27, 28
methyl propyl disulfide	94	1242	0.0729 ± 0.034	0.00728 ± 0.0044	6,* 9,* 29,* 21, 22, 23, 24, 26, 27, 28, 30
methyl 2-propenyl disulfide[t]	90	1296	0.0000819 ± 0.000019	0.00 ± 0	6,* 29,* 21, 23, 24, 26, 27, 30
methyl (E)-propenyl disulfide[t]	97	1292	0.0662 ± 0.026	0.00205 ± 0.00090	6*, 9*, 21, 22, 23, 24, 25, 26, 27, 28
ethyl 1-methylethyl disulfide[t]	95	1319	0.000452 ± 0.00019	0.00 ± 0	24
dipropyl disulfide	94	1387	0.197 ± 0.072	0.0409 ± 0.016	6,* 9,* 29,* 21, 22, 23, 24, 25, 26, 27, 28, 30
propyl (E)-propenyl disulfide[t]	90	1438	0.0437 ± 0.019	0.00452 ± 0.0021	6,* 9,* 21, 22, 23, 24, 25, 26, 28, 30
dimethyl trisulfide	97	1376	0.0432 ± 0.025	0.000296 ± 0.00014	6,* 9,* 21, 23, 24, 25, 26, 27, 28
diisopropyl trisulfide[t]	90	1656	0.0601 ± 0.033	0.0323 ± 0.014	21
propyl (E)-propenyl trisulfide[t]	95	1770	0.000555 ± 0.000080	0.000204 ± 0.000082	6,* 9,* 21, 23, 24, 25, 26, 27
3,4- or 2,4-dimethylthiophene[t]	97	1197	0.0338 ± 0.0072	0.0206 ± 0.0064	6,* 22, 24, 25, 28, 31*
propyl thioacetate[t]	94	1200	0.000886 ± 0.00014	0.00 ± 0	23, 26
3,5-diethyl-1,2,4-trithiolane[t]	95	1751	0.00231 ± 0.00030	0.000873 ± 0.00030	9,* 27
aldehydes					
pentanal	91	984	0.00 ± 0	0.330 ± 0.055	9*
hexanal	97	1111	1.53 ± 0.56	3.63 ± 0.61	9*, 26
heptanal	97	1197	0.00 ± 0	0.134 ± 0.023	
octanal	91	1299	0.00 ± 0	0.00441 ± 0.0013	
nonanal	98	1398	0.00 ± 0	0.00413 ± 0.0011	
(E)-2-butenal	95	1046	0.00 ± 0	0.231 ± 0.051	
(E)-2-pentenal	93	1147	0.00 ± 0	0.166 ± 0.017	
(E)-2-hexenal	98	1230	1.20 ± 0.73	0.168 ± 0.046	4,* 26
(E)-2-heptenal	96	1334	0.00 ± 0	0.156 ± 0.033	
(E)-2-octenal	96	1434	0.00 ± 0	0.0949 ± 0.021	
(E)-2-nonenal	91	1532	0.00474 ± 0.0018	0.00844 ± 0.0013	9*
(E)-2-decenal	90	1634	0.00 ± 0	0.00197 ± 0.00085	
2-methyl-(E)-2-butenal	94	1113	0.00 ± 0	0.717 ± 0.12	9,* 21, 31*
2-ethyl-(E)-2-butenal[t]	94	1166	0.00 ± 0	0.0263 ± 0.0045	
2-methyl-(E)-2-pentenal	97	1176	4.76 ± 0.83	0.203 ± 0.080	4,* 9,* 21, 22, 23, 24, 25, 26, 28, 31*
(E,Z)-2,4-heptadienal	91	1464	0.0600 ± 0.019	0.0181 ± 0.0045	
(E,E)-2,4-heptadienal	95	1490	0.0307 ± 0.0098	0.0137 ± 0.0079	9*
(E,Z)-2,4-nonadienal	93	1650	0.00 ± 0	0.0111 ± 0.0023	
(E,E)-2,4-nonadienal	94	1686	0.00 ± 0	0.0647 ± 0.018	
(E,Z)-2,4-decadienal	94	1746	0.0698 ± 0.027	0.211 ± 0.106	
(E,E)-2,4-decadienal	94	1789	0.129 ± 0.053	0.594 ± 0.24	9*
ketones					
2-octanone[t]	95	1297	0.00 ± 0	0.0250 ± 0.0085	
3-octanone	95	1272	0.00 ± 0	0.0165 ± 0.0013	
3-octen-2-one[t]	90	1345	0.00 ± 0	0.0327 ± 0.0060	
3,5-octadien-2-one[t]	93	1516	0.00 ± 0	0.00423 ± 0.00073	
6-undecanone[t]	93	1527	0.00 ± 0	0.00157 ± 0.00029	
β-ionone	98	1910	0.00 ± 0	0.00189 ± 0.00041	
alcohols					
1-pentanol	90	1274	0.00 ± 0	0.484 ± 0.061	
1-hexanol	90	1371	0.00 ± 0	0.184 ± 0.035	
1-octen-3-ol	90	1458	0.00 ± 0	0.0913 ± 0.010	
miscellaneous					
2,5-dimethylfuran[t]	95	958	0.00 ± 0	0.436 ± 0.034	
2-pentylfuran	91	1249	0.104 ± 0.024	0.0862 ± 0.020	

[a] Quality of the GC-MS identification. [b] Retention time index. [c] Standard deviation. [d] Figures correspond to references given in the literature cited section. *, investigations were carried out on leek. Nonmarked records were made on other *Allium* species. [t] Compound was identified by GC-MS data alone.

times the threshold. All of the (E)-2-monounsaturated aldehydes—except (E)-2-hexenal—increase during the months of storage (**Figure 4**), and only (E)-2-hexenal and (E)-2-nonenal are found in the fresh leek, whereas the others are formed during storage. After 12 months of storage, the concentration of (E)-2-octenal is ∼330 times the odor threshold and (E)-2-nonenal is present at ∼40 times the odor threshold with a maximum of 130 times the threshold at 6 months of storage [0.00034 ppm for (E)-2-octenal and 0.00023 ppm for (E)-2-nonenal (*17*)]. The concentration of (E)-2-hexenal is high in the fresh leeks, which agrees with the results of Ferary and Auger (*4*), who found this compound to be the predominant peak. The decline over time could indicate further conversion to (E)-2-hexenol or (Z)-2-hexenal, but no traces of these compounds were found in any of the samples.

(E,E)-2,4-Decadienal follows the same pattern as hexanal (**Figure 5**). The ratio of produced hexanal to (E,E)-2,4-decadienal suggests that the production of these aroma compounds during a frozen storage is due to not only autoxidation but also enzymatic activity, because autoxidation is not specific to one position on the fatty acid. This ratio also indicates that lipoxygenase isolated from leeks is producing hydroperoxides in both the C_{13} and C_9 positions on the fatty acids. LOX is more specific toward the 13-hydroperoxide though, because the concentration of hexanal is ∼10 times larger than that of (E,E)-2,4-decadienal. (E,E)-2,4-Nonadienal is also formed during frozen storage, whereas (E,E)-2,4-heptadienal diminishes. (E,E)-2,4-Nonadienal and (E,E)-2,4-decadienal have been shown to be very potent off-flavors, with threshold values of, respectively, 0.0017 and 0.045 ppb (*17*), which are far below the concentra-

Table 2. Statistical Calculations of Data Displayed in **Figures 2–6**

	storage period (months)[a]						
compound	0	1	2	4	6	9	12
2-methyl-2-pentenal	B	A	B	C	D	DE	E
3,4- or 2,4-dimethylthiophene	A	ABC	A	AB	BCD	D	CD
dipropyl disulfide	A	A	A	B	B	B	B
propyl (E)-propenyl disulfide	A	B	B	C	C	C	C
propyl (E)-propenyl trisulfide	C	AB	A	AB	B	C	D
pentanal	E	D	C	A	AC	A	A
hexanal	C	C	C	C	C	B	A
heptanal	C	C	C	C	B	B	A
octanal	C	C	C	C	B	B	A
nonanal	C	B	B	AB	B	AB	A
(E)-2-hexenal	A	BC	AB	BC	CD	CD	D
(E)-2-heptenal	E	D	BCD	CD	BC	A	AB
(E)-2-octenal	D	D	D	C	B	AB	A
(E)-2-nonenal	C	C	C	BC	A	AB	BC
(E)-2-decenal	C	C	C	C	B	B	A
(E,E)-2,4-heptadienal	A	A	A	A	AB	BC	C
(E,E)-2,4-nonadienal	D	D	C	C	B	A	A
(E,E)-2,4-decadienal	C	C	BC	BC	B	BC	A
LOX activity	AB	A	AB	AB	AB	BC	C
LOX lag phase	AB	B	AB	AB	B	AB	A

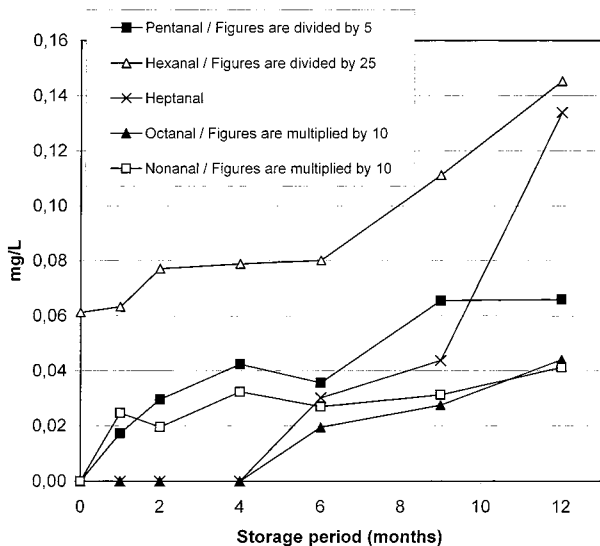[a] Different letters in a row indicate difference on a significance level of 5%.

Figure 3. Development of saturated aldehydes in leek slices during frozen storage. See **Table 2** for specification of statistical differences.

tion found in the leek slices after 12 months of frozen storage (**Table 1**). (E,E)-2,4-Heptadienal originates from the 12-hydroperoxide (18) and is consequently not produced by LOX, which might be the explanation for the different pattern of this aroma compound.

The formation of these aldehydes partly depends on the LOX-catalyzed oxidation of polyunsaturated fatty acids. Lipoxygenase showed a diminishing activity throughout the frozen storage, although ~25% of the original activity was still present after 12 months (**Figure 6**).

LOX activity is measured in a standard assay at optimal conditions of temperature and substrates, which gives no direct indication if the enzyme is actually active in frozen leek, only that it is able to show activity when the slices are crushed prior to the dynamic headspace analysis and oxygen is present in excess. When purged with nitrogen, the atmosphere becomes anaerobic within ~5 min, which means that LOX activity stops. Autoxidation of fatty acids could also produce these compounds (10), and both ways will cause accumulation in the tissue.
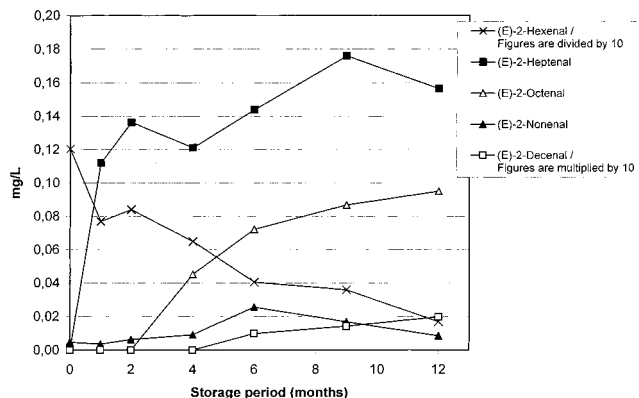

Figure 4. Development of monounsaturated (E)-2-aldehydes in leek slices during frozen storage. See **Table 2** for specification of statistical differences.
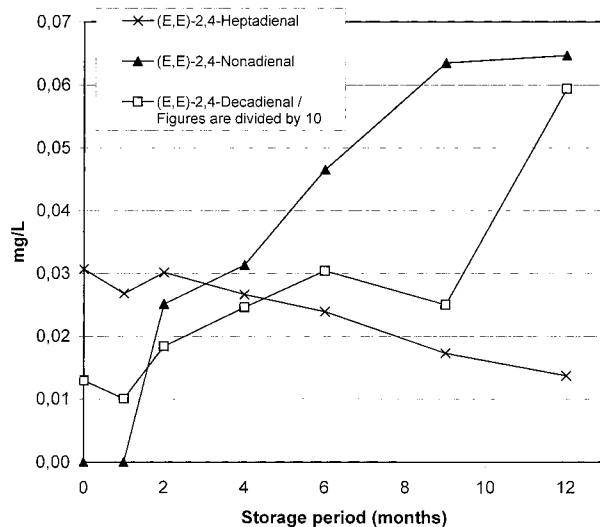

Figure 5. Development of diunsaturated (E,E)-2,4-aldehydes in leek slices during frozen storage. See **Table 2** for specification of statistical differences.
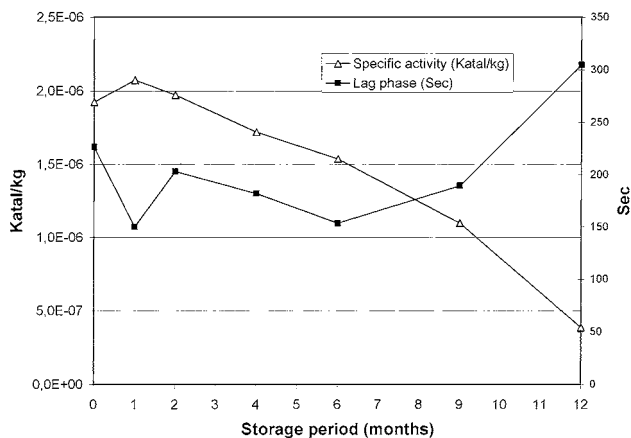

Figure 6. Specific activity (left y-axis) and lag phase (right y-axis) of lipoxygenase analyzed in leek slices during frozen storage. See **Table 2** for specification of statistical differences.

Autoxidation during the period of tempering and purging in the aroma analysis should be the same at every sampling time during storage, whereas lipoxygenase activity changes; it would be interesting to separate the two processes by inactivating the enzymes before aroma analysis. A number of ketones were also

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found to develop during frozen storage, which could originate from autoxidation of polyunsaturated fatty acids (*10*).

Lipoxygenase contains 1 mol of non-heme iron per mole of enzyme, which is essential for catalytic activity (*19*). To catalyze a reaction, LOX must be in the oxidized form (Fe^{3+}), which only ~1% of the native enzyme is (*20*). Oxidation is usually done by its own lipid hydroperoxide product; consequently, a lag phase is present until total activity is reached. **Figure 6** shows the length of the lag phase throughout the frozen storage, which increases. There are several reasons to believe that LOX would be activated in the tissue when the leek slices were stored for a longer period. There is the possibility that more and more lipid hydroperoxides would generate through storage because compartmentation of lipoxygenase and the polyunsaturated fatty acids could be ruined over a freezing period due to loss of cell structure during the growth of ice crystals. Also, more free fatty acids could be available due to lipase activity, and reactions could occur in the phase of liquid water during frozen storage. These results and the decreasing activity indicate a considerable loss of LOX in the tissue during frozen storage.

Table 1 states which compounds have been found by other authors, and it is noticeable that all of the sulfur compounds except methyl pentyl sulfide are in agreement with other investigations performed on *Allium* species. Most of the compounds originating from the LOX pathway and/or the autoxidation route have been found for the first time by this work. This is closely related to the fact that these compounds generate during frozen storage, and all of the studies used for comparison, except ref *9,* have been performed on fresh leeks.

CONCLUSION

The aroma profile of leek slices undergoes a remarkable alteration during 12 months of frozen storage when compared with freshly cut leeks. There is a substantial decrease of the characteristic sulfur compounds, and the concentration of saturated and unsaturated aldehydes increases as an expression of lipoxygenase activity and/or autoxidation of polyunsaturated fatty acids. The catalytic activity of LOX in frozen leek is present throughout a storage term of 12 months, although it is declining and the lag phase is slightly increasing. When leeks are not blanched prior to freezing, the metabolism of fatty acids could be a limitation of the shelf life of the product due to the formation of off-flavor, and there is no doubt that the flavor of frozen leeks stored for 4−6 months or more is quite different from that of fresh leeks.

ABBREVIATIONS USED

LOX, lipoxygenase; FL, concentration in fresh leek; 12M, concentration after 12 months of frozen storage.

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