JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Formation of Aroma Compounds during Long-Term Frozen Storage of Unblanched Leek (*Allium ampeloprasum* Var. *Bulga*) as Affected by Packaging Atmosphere and Slice Thickness

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Content of aroma compounds and catalytic activity of lipoxygenase (LOX), hydroperoxide lyase (HPL), and alcohol dehydrogenase (ADH) were analyzed in 4- and 15-mm unblanched leek slices packed in atmospheric air (4- and 15-mm) or 100% nitrogen (N) (only 15-mm) seven times during 12 months of frozen storage (12M). Total amount of sulfur compounds was influenced by storage time, slice thickness, and atmosphere (concentration in fresh 4-mm slices = 17.8 mg/L, 4-mm 12M = 3.48 mg/L, fresh 15-mm slices = 2.48 mg/L, 15-mm 12M = 0.418 mg/L and 15-mm N 12M = 1.81 mg/L). The 4-mm slices significantly developed the most aldehydes after 12M (total amount = 9.28 mg/L) compared to 15-mm 12M (6.49 mg/L) and 15-mm N 12M (4.33 mg/L). LOX activity is positively influenced by nitrogen packaging, and HPL activity is influenced by slice thickness, whereas ADH is unaffected by both parameters.

KEYWORDS: Leek; flavor; frozen storage; lipoxygenase; hydroperoxide lyase; alcohol dehydrogenase; nitrogen packaging; slice thickness

INTRODUCTION

The aroma of freshly cut leek and other *Allium* species is dominated by numerous sulfur-containing volatile compounds originating from the alliinase- (EC 4.4.1.4) catalyzed decomposition of the odorless nonvolatile precursors *S*-alk(en)-ylcysteine sulfoxides (*1*, *2*).

The products, sulfenic acids, are highly reactive (*3*) and will quickly combine to form thiosulfinates. Thiosulfinates are responsible for the odor of freshly cut leeks (*3*, *4*), but as they are relatively unstable (*1*, *5*), they will rearrange to form polysulfides and thiosulfonates. As thiosulfonates are transformed to the corresponding monosulfides, the final products of the reaction will be a combination of mono- and polysulfides with all of the possible combinations of the alk(en)yl radicals. Prior investigations (*6*) have shown that these sulfur compounds decline during frozen storage of leek slices.

The lipoxygenase pathway also contributes to the aroma formation in fresh and especially in stored leeks. Lipoxygenase (EC 1.13.11.12) (LOX) catalyzes the formation of hydroperoxy derivatives of polyunsaturated fatty acids with a *cis*,*cis*-pentadiene moiety (*7*, *8*) under the consumption of dioxygen. Volatile aldehydes are produced by the action of hydroperoxide lyase (HPL) upon the formed hydroperoxides (*7*, *9*). All of these aldehydes, both saturated and nonsaturated, can be further metabolized by alcohol dehydrogenase (EC 1.1.1.1) (ADH) to the corresponding alcohols (*9*).

This formation of mainly aldehydes and alcohols may contribute to the aroma of fresh leeks but will probably appear as off-flavors when present in larger amounts (*6*, *10*). This is not very pronounced in the fresh leek because of the pungency of thiosulfinates and thiopropanal-*S*-oxide (*5*). 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, both because of the increasing amount of these compounds and because of the diminishing content of sulfur compounds (*6*, *10*). These compounds derived from fatty acids can also be generated by autoxidation during the storage period (*11*), and both pathways will result in accumulation of the products in the tissue.

Previous investigations of unblanched frozen leek slices (*6*) have shown that the storage period has a great influence on the aroma compounds in the leek slices and that the concentration of saturated and unsaturated aldehydes increases effectively during the storage. This paper deals with the impact of changing the headspace in the package of the frozen leek slices from atmospheric air to 100% nitrogen, which to our knowledge has not been investigated before. This could prevent oxidation during the frozen storage, which will result in less accumulation of off flavor in the leek slices. Also, the influence of slice thickness

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10.1021/jf0306183 CCC: $27.50
Published on Web 02/11/2004

on the development of aroma compounds during frozen storage is investigated.

MATERIALS AND METHODS

Plant Material. Leeks (*Allium ampeloprasum* var. *Bulga*) were harvested fully matured at Funen, Denmark in October 2000 and used for the experiment immediately after.

Chemicals. 1-Propanethiol, dimethyl disulfide, methyl propyl disulfide, dipropyl disulfide, dimethyl trisulfide, 2,5-dimethylthiophene, propanal, butanal, pentanal, hexanal, heptanal, octanal, nonanal, decanal, (*E*)-2-butenal, (*E*)-2-pentenal, (*E*)-2-hexenal, (*E*)-2-heptenal, (*E*)-2-octenal, 2-methyl-(*E*)-2-butenal, 2-methyl-(*E*)-2-pentenal, (*E*,*E*)-2,4-heptadienal, (*E*,*E*)-2,4-nonadienal, 3-octanone, 1-pentanol, 1-hexanol, 1-octen-3-ol, and 2-pentyl furan were bought commercially from Sigma-Aldrich, Copenhagen, Denmark. (*E*,*Z*)-2,4-Heptadienal was occurring as an impurity in (*E*,*E*)-2,4-heptadienal. All other chemicals, except 9-(S)-hydroperoxy-(10E,12Z)-octadecadienoic acid, were of analytical grade and were bought commercially from Sigma-Aldrich.

Synthesis of 9-(*S*)-Hydroperoxy-(10*E*,12*Z*)-octadecadienoic Acid (9-HPODE). Sodium linoleate (1.25 mL, 10 mM), 4.75 mL of air saturated 50 mM potassium phosphate buffer (pH 7.0), and 1.50 mL of potato tuber lipoxygenase solution were mixed then agitated and purged with oxygen for 30 min. The solution was checked for absorbance at 234 nm to determine the content of 9-HPODE. According to Galliard and Phillips (*12*), potato tuber LOX almost exclusively converts linoleic acid into 9-HPODE.

Preparation of Potato Tuber Lipoxygenase Solution for Synthesis. Grated potato tubers (50 g) and 50 mL of tap water were homogenized for 30 s in a Waring commercial blender and filtrated through a paper filter. A 2-mL aliquot of the liquid was applied to a DEAD-Cellulose C545 column (anion exchanger) followed by 6.0 mL of 50 mM potassium phosphate buffer (pH 7.0). Two fractions of 4.0 mL were collected; both were checked for activity, and the second one was used as the lipoxygenase solution.

Sample Preparation. The white part of the leek stem was cut into 4-mm slices or 15-mm slices and frozen immediately after in a blast freezer at −20 °C for 10 min, so that the center temperature reached −20 °C. The slices were packed in airtight glass jars with atmospheric air or 100% nitrogen (only 15-mm slices) as headspace and kept at −20 °C in the dark until analysis. Fresh Pax oxygen absorbers (Type R 50 CC) from Multisorb Technologies, Inc. Buffalo, NY were added to the glasses with 100% nitrogen.

Experimental Design. Two individual samples of 4-mm slices and 15-mm slices were analyzed at harvest time, and subsequently, two individual samples of 4,- 15-, and 15-mm N were analyzed after 1, 2, 4, 6, 9, and 12 months of storage. Each sample was analyzed for composition of atmosphere in the glass jars, catalytic activity of lipoxygenase, hydroperoxide lyase, and alcohol dehydrogenase, and content of aroma compounds in three replicates.

Gas Analysis. The O_2, CO_2, and N_2 concentrations in the glass jars were determined by a Gaspace 2 analyzer from Systech Instruments Ltd, Oxon, UK.

Preparation of Enzyme Extract. Enzyme extract was made by mixing 100 g of frozen leek slices with 100 mL of potassium phosphate buffer (50 mM, pH 7.0 added 0.1% Triton X-100) for 120 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 until 35.0 mL was collected, and kept on ice until time of analysis.

Lipoxygenase Assay. LOX was assayed in a continuous assay by measuring consumption of initial dioxygen by an YSI 5100 dissolved oxygen meter (YSI Inc., Yellow Springs, OH) at 30 °C, 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 dioxygen) followed by addition of sodium dithionite (0% dissolved oxygen). To determine lipoxygenase activity, 27.2 mL of air-saturated tempered phosphate buffer (0.2 M, pH = 6.0) and 3.9 mL of enzyme extract was mixed in a 33-mL conical flask, and the measuring was started. After 30 s, the reaction was initiated by adding 1.9 mL of sodium linoleate solution (10 mM). Dioxygen was monitored over 20 min, measuring dioxygen content every second.

Blank samples were run on buffer added substrate or enzyme extract.

Calculation of LOX Activity. LOX activity was calculated as katals per kg of protein, and katals were defined as moles of O_2 per second. This was done under the assumption that air-saturated phosphate buffer (0.2 M, pH 6.0) at 30 °C with a salt strength of 12.40 g/L contains 7.11 mg O_2/L (*13*). To determine the activity the dioxygen level (mmol O_2) was plotted against time and the steepest slope (mmol O_2/s) on the curve was found using continuous linear regression over 20 s at a time.

Hydroperoxide Lyase Assay. HPL was assayed in a continuous coupled assay modified after (*14*). ADH converts the products of HPL's reaction on hydroperoxides, aldehydes, under the oxidation of NADH, which can be monitored spectrophotometrically at 340 nm.

Phosphate buffer (1.8 mL, 0.2 M, pH = 6.0), 300 μL of 2.2 mM NADH, 200 μL of ADH (150 units in 100 μL) and 300 μL of enzyme extract (filtrated through 1.2-μm and 0.45-μm filters from Orange Scientific, Braine-l'Alleud, Belgium) were transferred to a thermostatic (30 °C) 1-cm cuvette with a magnetic stirrer, and the measuring was started. After 30 s, 400 μL of 9-HPODE was added. The reaction was monitored over 600 s.

Blank samples were run on buffer, NADH, and ADH added substrate or enzyme extract.

Calculation of HPL Activity. HPL activity was calculated as katals per kg of protein and katals were defined as moles consumed of hydroperoxide/s. To determine the activity, the absorbance was plotted against time, and the steepest slope (absorbance/s) on the curve was found using continuous linear regression over 5 s at a time. Absorbance was converted to moles of hydroperoxide by the assumption that each mole of hydroperoxide metabolized produced one mole of aldehyde. Aldehydes are converted by alcohol dehydrogenase by the consumption of NADH 1:1. ϵ of NADH at 340 nm is 6220 L/mol · cm (*14*).

Alcohol Dehydrogenase Assay. ADH was assayed in a continuous assay by following the oxidation of NADH spectrophotometrically at 340 nm when adding hexanal.

Borate buffer (1.4 mL, 0.05 M, pH = 9.0), 300 μL of 2.2 mM NADH and 1000 μL of enzyme extract (filtrated through 1.2-μm and 0.45-μm filters from Orange Scientific,') were transferred to a thermostatic (30 °C) 1-cm cuvette with a magnetic stirrer, and the measuring was started. After 30 s, 300 μL of hexanal (100 ppm) was added. The reaction was monitored over 600 s.

Blank samples were run on buffer and NADH added substrate or enzyme extract.

Calculation of ADH Activity. Alcohol dehydrogenase activity was calculated as katals per kg of protein, and katals were defined as moles of hexanal/s. Moles of hexanal were calculated on the basis of NADH under the assumption that hexanal and NADH were metabolized 1:1 and by the conditions described under calculation of HPL activity.

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. Fresh or frozen leek slices (100 g) were crushed with 150 mL of tap water and 4 mL of internal standard (50 ppm of 4-methyl-1-pentanol in tap water) for 120 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 250 mg of Tenax GR (mesh size = 60/80, Buchem bv, Apeldoorn, The Nederlands) for 45 min with a nitrogen flow of 75 mL/min and agitation (200 rpm).

Desorption of aroma compounds was done thermally by an ATD 400 automatic thermal desorption system (Perkin-Elmer, Bucks, England). Desorption temperature of the trap to the cold trap (contains 30 mg Tenax GR, 5 °C) was 250 °C for 15 min with a helium flow of 60 mL/min. Desorption temperature of the cold trap was 300 °C for 4 min, with a helium flow of 31 mL/min and an outlet split ratio of 1:30. Separation was performed by a GC-MS (HP G1800 A GCD system) with the following conditions: column, DB Wax from J&W Scientific, CA (30-m × 0.25-mm i.d. × 0.25-μm film thickness); carrier gas,

Table 1. Atmosphere Composition in % in the Glass Jars during Storage of Frozen Leek Slices

storage period (months)	4 mm			15 mm			15 mm N		
	O_2	CO_2	N_2	O_2	CO_2	N_2	O_2	CO_2	N_2
1	18.5	3.2	78.3	18.7	3.5	77.8	0.5	2.5	97.0
2	19.1	2.2	78.7	18.6	3.8	77.6	0.9	2.0	97.1
4	18.1	1.8	80.1	17.4	3.5	79.1	0.9	1.8	97.3
6	17.7	1.8	80.5	15.7	4.9	79.4	0.1	2.2	97.7
9	17.1	1.8	81.1	14.8	4.5	80.7	0.4	1.7	97.9
12	16.4	2.7	80.9	13.6	6.4	80.0	0.1	2.0	97.9

helium; start flow, 1 mL/min (constant); split ratio, none; column pressure (constant), 48 kPa; oven program, 45 °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 (ionization energy, 70 eV), and the *m*/*z* (mass/charge) ratio ranged between 10 and 425. Identification was done by probability-based matching with mass spectra in the G1035A Wiley library (Hewlett-Packard). Identity was confirmed by checking with mass spectra and retention indices obtained in the laboratory from reference compounds for the following compounds: 1-propanethiol, dimethyl disulfide, methyl propyl disulfide, dipropyl disulfide, dimethyl trisulfide, 2,5-dimethylthiophene, propanal, butanal, pentanal, hexanal, heptanal, octanal, nonanal, decanal, (*E*)-2-butenal, (*E*)-2-pentenal, (*E*)-2-hexenal, (*E*)-2-heptenal, (*E*)-2-octenal, 2-methyl-(*E*)-2-butenal, 2-methyl-(*E*)-2-pentenal, (*E*,*Z*)-2,4-heptadienal, (*E*,*E*)-2,4-heptadienal, (*E*,*E*)-2,4-nonadienal, 3-octanone, 1-pentanol, 1-hexanol, 1-octen-3-ol, and 2-pentyl furan.

Quantification. A 10-μL aliquot of each of the above listed reference compounds was dissolved in 20 mL of 96% ethanol and diluted with tap water into four appropriate concentrations depending on the concentration of the compounds in the leeks. Sucrose (1.3%), which equals the sugar content of leeks, was added to the final solutions. Dynamic headspace analysis performed on 400 mL of the solutions added 4 mL of internal standard with the same flow, time, and temperature conditions as applied to the leek samples. Each reference compound was analyzed in triplicate in all four concentrations. The obtained peak area divided by the peak area of the internal standard was used to calculate the concentration of the same compound in the leeks from the peak area of the compound divided by the peak area of the internal standard. Methyl 2-propenyl disulfide, methyl propenyl disulfide, and ethyl 1-methylethyl disulfide were quantified after the obtained peak area of methyl propyl disulfide, propyl 2-propenyl disulfide; propyl propenyl disulfide after dipropyl disulfide, methyl propyl trisulfide, diisopropyl trisulfide; propyl propenyl trisulfide after dimethyl trisulfide; 3,4- or 2,4-dimethyl thiophene after 2,5-dimethyl thiophene; 2-ethyl-(*E*)-2-butenal after 2-methyl-(*E*)-2-pentenal, 3-octen-2-one; 3,5-octadiene-2-one after 3-octanone; and 2,5-dimethyl furan after 2-pentyl furan

The retention time indices (RTI) of the volatile compounds were calculated with a mixture of hydrocarbons (C_9−C_{26}) as references, according to the method of (*15*).

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

RESULTS AND DISCUSSION

The actual composition of the atmosphere in the glass jars at each sampling time is displayed in **Table 1**. The nitrogen packaging keeps very constant at 97−98% N_2 and below 1% of O_2. The 4- and 15-mm slices both modify the atmosphere in the jars packed with atmospheric air. The 15-mm slices are most effective in changing the atmosphere, which ends at 6.4% CO_2 and 13.6% O_2, suggesting that respiration occurs in the leek slices during the frozen storage and that the degree of intact tissue per slice has an impact on this activity.

A complete overview over the aroma compounds detected in leek slices, fresh and after 12 months of frozen storage, is given in **Table 2** for all three treatments (4-, 15-, and 15-mm N slices). Of the detected sulfur compounds, the aroma is dominated by dipropyl disulfide, especially in the fresh leek slices, which is consistent with what Keusgen et al. (*16*) found in various *Allium* hybrids. There is a noticeable effect of the slicing thickness as the 4-mm fresh cut slices produces significantly more of all of the sulfur compounds compared to the other treatments shown in **Table 2**, the exceptions being equal amounts of dimethyl disulfide and 2,5-dimethyl thiophene produced in 4- and 15-mm fresh slices and diisopropyl trisulfide produced in 4- and 15-mm N 12M slices. The sulfur volatiles are not produced in the intact cells because of compartmentation of alliinase in the vacuole and the cysteine sulfoxides in the cytoplasm (*2*, *17*), but during the cleaning and slicing process, the formation of sulfur compounds starts. The 4-mm slices have a larger percent of disrupted cells per weight unit than the 15-mm slices have, which can explain this observed difference. During the headspace collection of volatiles, though, formation of sulfur compounds might be possible because the slices are totally crushed during the sample preparation procedure. Purging with nitrogen quickly creates anaerobic conditions in the leek slurry, but alliinase is not dependent on oxygen being present as lipoxygenase is.

This observed effect of the slice thickness is not only valid for the fresh leek slices, as there are also significant differences between slice thicknesses when the leek slices have been stored frozen for 12 months. For many sulfur compounds, such as dipropyl disulfide, 4-mm slices have a tendency to have the largest concentration throughout the storage period. The same pattern is found for methyl propyl disulfide, ethyl 1-methylethyl disulfide, propyl propenyl disulfide, propyl 2-propenyl disulfide, and diisopropyl trisulfide. For 4-mm slices all of the sulfur compounds decreased effectually during frozen storage, which is in agreement with what is already found for 15-mm slices (*6*). In the present study, 15-mm slices also show significant changes for most of the sulfur compounds during 12 months of frozen storage (e.g., dimethyl disulfide (**Figure 1**) decreases significantly). **Table 3** presents the statistical differences of data displayed in **Figures 1**−**5**.

In contrast to our previous investigations (*6*), this experiment shows that all of the sulfur compounds are still present after 12 months of frozen storage in both slice thicknesses and in the nitrogen packaging. While most of the compounds follow the pattern of **Figure 1**, 2,5-dimethyl thiophene is present at a constant concentration from 2 to 9 months of storage, except for a high value found after 1 month of storage, and is only decreased during the last 3 months of storage.

When comparing the total amount of sulfur compounds, the effect of frozen storage stands out clearly. The 4-mm slices alter from 17763 μg/L in the fresh leeks to 3475 μg/L after 12 months of frozen storage, and the 15-mm fresh leek slices contain 2480 μg/L, whereas leek slices frozen for 12 months contain 418 μg/L (**Table 2**), which in both cases are significant changes.

Nitrogen packaging has a great influence on the keeping of the sulfur compounds (**Table 2**). Half of the sulfur compounds are found in equal concentrations after 12 months of frozen storage under nitrogen compared to the fresh 15-mm slices, and two even in higher concentrations. Diisopropyl trisulfide is present in 425 μg/L, which is an unexpected high part of the total amount (1809 μg/L) of sulfur compounds that were

Table 2. Aroma Compounds Found in Fresh Leek and in Leeks Frozen for 12 Months (12 M)[a]

compound	RTI[b]	4-mm fresh leek	4-mm 12 M	15-mm fresh leek	15-mm 12 M	15-mm N[c] 12 M
			Sulfur Compounds			
1-propanethiol	857	260 ± 197	36.2 ± 6.88	54.7 ± 38.5	19.8 ± 7.15	55.3 ± 24.4
dimethyl disulfide	1086	170 ± 55.4	11.0 ± 4.50	137 ± 130	2.21 ± 1.77	5.00 ± 3.32
methyl propyl disulfide	1242	1314 ± 586	318 ± 104	257 ± 156	27.4 ± 19.5	129 ± 33.9
methyl 2-propenyl disulfide[e]	1296	0.635 ± 0.231	0.0826 ± 0.0237	0.208 ± 0.0861	0.0131 ± 0.0141	0.0418 ± 0.00869
methyl propenyl disulfide[e,f]	1263	312 ± 110	4.69 ± 1.19	85.3 ± 26.8	2.16 ± 1.45	7.58 ± 1.98
methyl propenyl disulfide[e,f]	1292	1225 ± 443	19.1 ± 5.87	250 ± 72.8	6.94 ± 4.20	21.5 ± 5.98
ethyl 1-methylethyl disulfide[e]	1319	7.87 ± 3.21	4.35 ± 1.05	0.993 ± 0.349	0.595 ± 0.247	2.38 ± 0.643
dipropyl disulfide	1387	11076 ± 4249	2630 ± 590	1250 ± 320	208 ± 67.3	966 ± 260
propyl 2-propenyl disulfide[e]	1436	1.64 ± 0.687	0.486 ± 0.138	0.124 ± 0.0521	0.0137 ± 0.0151	0.257 ± 0.415
propyl propenyl disulfide[e,f]	1422	115 ± 62.8	14.8 ± 3.47	20.3 ± 8.65	4.42 ± 1.60	15.5 ± 4.69
propyl propenyl disulfide[e,f]	1438	1520 ± 720	94.2 ± 22.7	153 ± 89.0	37.7 ± 21.8	58.3 ± 22.7
dimethyl trisulfide	1376	231 ± 94.2	1.70 ± 0.604	40.9 ± 10.5	0.573 ± 0.806	4.76 ± 1.26
methyl propyl trisulfide[e]	1531	361 ± 178	21.7 ± 6.17	44.3 ± 19.2	5.00 ± 4.25	30.6 ± 11.1
diisopropyl trisulfide[d]	1656	549 ± 239	255 ± 55.2	84.3 ± 56.8	50.2 ± 20.8	425 ± 75.9
propyl propenyl trisulfide[e,f]	1781	20.8 ± 5.06	0.780 ± 0.139	2.48 ± 1.01	0.836 ± 0.252	2.97 ± 0.686
propyl propenyl trisulfide[e,f]	1770	24.8 ± 4.94	1.19 ± 0.238	3.32 ± 1.53	0.886 ± 0.291	3.21 ± 0.709
2,5-dimethyl thiophene	1190	18.9 ± 8.75	1.91 ± 0.382	14.0 ± 5.26	3.00 ± 1.49	6.42 ± 1.22
3,4- or 2,4-dimethyl thiophene[e]	1253	557 ± 484	59.9 ± 11.1	82.7 ± 48.9	47.7 ± 10.5	76.2 ± 15.9
total of sulfur compounds		17763 ± 7340	3475 ± 769	2480 ± 583	418 ± 128	1809 ± 444
			Aldehydes			
propanal	762	8118 ± 1980	1697 ± 630	3683 ± 720	2811 ± 1273	4348 ± 635
butanal	867	0.00 ± 0	25.5 ± 6.19	0.00 ± 0	41.0 ± 11.8	20.1 ± 5.81
pentanal	984	225 ± 104	1426 ± 337	144 ± 15.7	770 ± 41.0	351 ± 759
hexanal	1111	1349 ± 140	4256 ± 1099	1191 ± 397	3178 ± 195	1191 ± 279
heptanal	1197	0.00 ± 0	115 ± 26.4	0.00 ± 0	85.0 ± 3.73	39.5 ± 4.70
octanal	1299	0.00 ± 0	15.9 ± 3.50	0.00 ± 0	8.03 ± 1.23	0.154 ± 0.128
nonanal	1398	0.561 ± 0.364	4.32 ± 3.30	0.0722 ± 0.0179	4.96 ± 0.597	2.71 ± 0.619
decanal	1510	0.00 ± 0	1.14 ± 0.622	0.00 ± 0	0.881 ± 0.393	0.536 ± 0.273
(*E*)–2-butenal	1046	0.00 ± 0	781 ± 237	0.00 ± 0	415 ± 153	264 ± 75.5
(*E*)–2-pentenal	1147	0.00 ± 0	149 ± 39.6	0.00 ± 0	111 ± 26.5	178 ± 12.8
(*E*)–2-hexenal	1230	1275 ± 145	22.3 ± 12.2	1072 ± 881	39.1 ± 23.7	82.0 ± 17.1
(*E*)–2-heptenal	1334	219 ± 34.1	85.5 ± 16.6	122 ± 35.1	81.4 ± 4.47	85.4 ± 7.67
(*E*)–2-octenal	1434	0.783 ± 0.511	0.435 ± 0.394	0.838 ± 1.10	49.1 ± 3.70	18.2 ± 11.6
2-methyl-(*E*)–2-butenal	1113	0.00 ± 0	2349 ± 596	0.00 ± 0	1660 ± 258	2013 ± 283
2-ethyl-(*E*)–2-butenal[e]	1166	0.00 ± 0	17.2 ± 11.5	0.00 ± 0	24.5 ± 8.92	15.5 ± 24.1
2-methyl-(*E*)–2-pentenal	1176	7747 ± 2280	91.9 ± 22.1	5061 ± 954	152 ± 100	506 ± 71.9
(*E*,*Z*)–2,4-heptadienal	1464	358 ± 38.9	6.63 ± 5.31	141 ± 77.7	10.9 ± 4.50	40.5 ± 8.66
(*E*,*E*)–2,4-heptadienal	1490	175 ± 57.2	13.4 ± 11.0	45.2 ± 20.9	9.84 ± 1.97	30.1 ± 2.04
(*E*,*E*)–2,4-nonadienal	1686	0.00 ± 0	12.9 ± 6.08	0.00 ± 0	3.85 ± 6.67	2.20 ± 2.36
total of aldehydes[d]		3602 ± 334	9281 ± 2310	2717 ± 1380	6489 ± 358	4333 ± 553
			Ketones			
3-octanone	1272	0.00 ± 0	11.0 ± 3.30	0.00 ± 0	11.9 ± 2.31	1.38 ± 1.08
3-octen-2-one[e]	1345	0.00 ± 0	7.62 ± 4.20	0.00 ± 0	5.30 ± 0.362	0.487 ± 0.511
3,5-octadiene-2-one[e]	1516	0.00 ± 0	7.83 ± 2.29	0.00 ± 0	4.41 ± 1.43	1.34 ± 0.425
total of ketones		0.00 ± 0	26.5 ± 8.70	0.00 ± 0	21.6 ± 2.35	3.20 ± 1.20
			Alcohols			
1-pentanol	1274	0.00 ± 0	356 ± 87.0	0.00 ± 0	299 ± 40.9	207 ± 22.3
1-hexanol	1371	0.00 ± 0	56.1 ± 35.8	0.00 ± 0	172 ± 80.1	17.4 ± 8.38
1-octen-3-ol	1458	0.00 ± 0	57.1 ± 14.1	0.00 ± 0	46.3 ± 2.60	10.9 ± 7.11
total of alcohols		0.00 ± 0	469 ± 106	0.00 ± 0	517 ± 112	235 ± 29.7
			Furans			
2,5-dimethyl furan[e]	958	0.00 ± 0	4.93 ± 1.30	0.00 ± 0	6.37 ± 2.20	0.261 ± 0.304
2-pentyl furan	1249	55.5 ± 23.3	17.0 ± 3.73	36.3 ± 11.3	17.1 ± 4.04	29.7 ± 6.50
total of furans		55.5 ± 23.3	22.0 ± 4.95	36.3 ± 11.3	23.5 ± 4.87	29.9 ± 6.43

[a] All values except RTI are in μg/L ± standard deviation. [b] Retention time index. [c] N = packed in nitrogen. [d] Except for propanal and 2-methyl-2-pentenal. [e] Compound was identified by GC-MS alone and quantified by peak area of another compound, see Materials and Methods for details. [f] (*Z*) and (*E*) isomers.

detected in the leek slices packed under nitrogen. Most of the sulfur compounds are found in significantly higher concentrations in the nitrogen packed leek slices compared to the 12 months frozen storage of 15-mm slices. Nitrogen packaging prevents the respiratory activity that is viable at −20 °C (**Table 1**), and possibly at the same time the metabolic degradation of sulfur compounds is more or less prevented, resulting in the positive aroma keeping of the sulfur compounds.

Aldehydes show a different development during storage, 9 out of 19 are not found at all in the fresh leek slices, regardless of slice thickness; this applies to butanal, heptanal, octanal, decanal, (*E*)-2-butenal, (*E*)-2-pentenal, 2-methyl-(*E*)-2-butenal, 2-ethyl-(*E*)-2-butenal, and (*E*,*E*)-nonadienal, and most of the others are present only in very low concentrations in the fresh tissue (**Table 2**). The exceptions are propanal, (*E*)-2-hexenal, (*E*)-2-heptenal, 2-methyl-(*E*)-2-pentenal, and both 2,4-heptadienals, which are all exhibiting the same pattern as dimethyl disulfide (**Figure 1**). (*E*)-2-Hexenal is found by others (*3*) to be present in large amounts in fresh leek and is shown to follow the typical pattern of sulfur compounds (*6*). Propanal and 2-methyl-(*E*)-2-pentenal are both breakdown products from thiopropanal-*S*-oxide (the lachrymatory factor) (*18*), which is not detectable with our GC-method (*6*, *19*). Of the detected aldehydes in the fresh leek slices, there are significant differ-

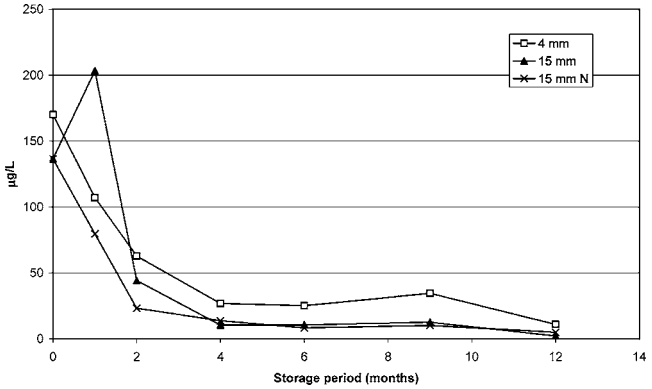

Figure 1. Development of dimethyl disulfide in leek slices during frozen storage. See **Table 3** for specification of statistical differences within one treatment.

Table 3. Statistical Calculations of Data Displayed in **Figures 1–5**

processing method	storage period (months)[a]						
	0	1	2	4	6	9	12
			Figure 1				
			Dimethyl Disulfide				
4-mm	A	B	C	D	D	CD	D
15-mm	B	A	C	C	C	C	C
15-mm N	A	AB	BC	C	C	C	C
			Figure 2				
			Hexanal				
4-mm	E	D	B	A	C		
15-mm	D	CD	CD	C	B	A	B
15-mm N	B	B	B	B	B	A	B
			Figure 3				
			LOX Activity				
4-mm	A	B	C	C	C	C	C
15-mm	A	B	E	C	DE	CD	CDE
15-mm N	B	C	DE	E	CD	A	C
			Figure 4				
			HPL Activity				
4-mm	A	A	A	B	B	B	A
15-mm	AB	A	BC	C	C	C	AB
15-mm N	A	A	A	BC	AB	C	A
			Figure 5				
			ADH Activity				
4-mm	A	AB	A	BCD	CD	BC	D
15-mm	A	A	AB	ABC	BCD	CD	D
15-mm N	A	A	A	B	B	B	B

[a] Different letters in a row indicates difference on a significance level of 5%. Letters cannot be compared among rows.

ences in concentration between the two thicknesses for 6 out of the 10 detected aldehydes, namely propanal, nonanal, (*E*)-2-heptenal, 2-methyl-(*E*)-2-pentenal, and both 2,4-heptadienals, where 4-mm slices generate the largest concentrations.

During frozen storage, slice thickness has a strong impact on development of off flavor, as many of the aldehydes are found in the highest concentration in 4-mm slices (**Table 2**). This is found after 12 months of frozen storage, but applies to the whole storage period. **Figure 2** shows the development of hexanal, which resembles those of pentanal, octanal, (*E*)-2-butenal, (*E*)-2-pentenal, (*E*)-2-heptenal, and (*E*,*E*)-2,4-nonadienal. Compartmentation of LOX in the cytosol and its substrate (fatty acids) in the cell membranes makes the basis for enzymatic activity of LOX and production of aldehydes during frozen storage more favorable in the 4-mm slices because of a larger degree of disrupted cells per weight unit that in the 15-mm slices.

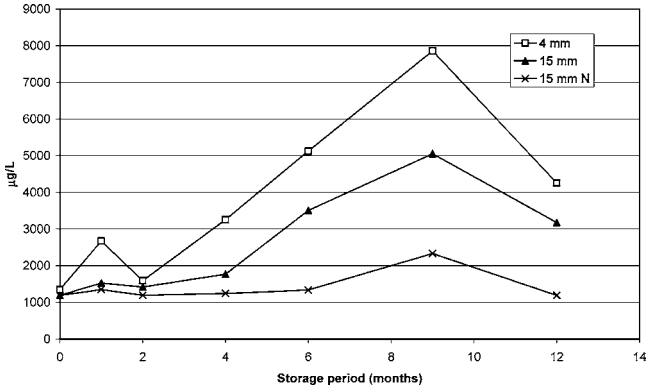

Figure 2. Development of hexanal in leek slices during frozen storage. See **Table 3** for specification of statistical differences within one treatment.

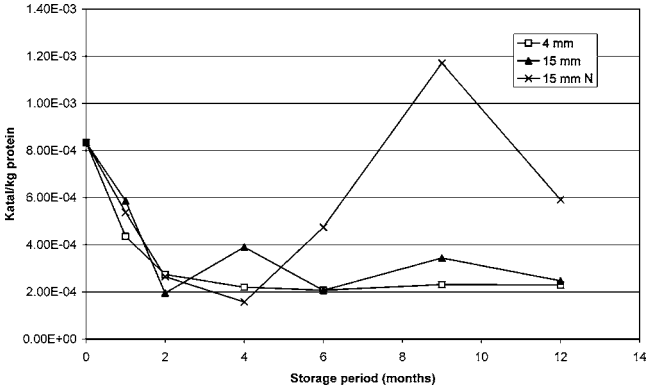

Figure 3. Specific activity of LOX analyzed in leek slices during frozen storage. See **Table 3** for specification of statistical differences within one treatment.

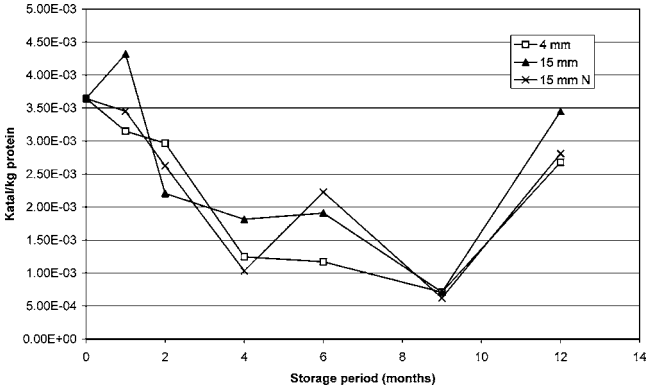

Figure 4. Specific activity of HPL analyzed in leek slices during frozen storage. See **Table 3** for specification of statistical differences within one504 treatment.

Nitrogen packaging is to some extent preventing formation of off-flavor in the leek slices during frozen storage. After 12 months of frozen storage under nitrogen, 11 out of 19 aldehyde compounds found are in the same level as the fresh leek slices or in a lower concentration than the 15-mm slices stored for 12 months. Hexanal (**Figure 2**) is kept almost constant during storage, when nitrogen is present in the glass jar, whereas it develops effectually in atmospheric air headspace. This is also found for butanal, pentanal, heptanal, octanal, (*E*,*E*)-2,4-nonadienal, and hexanol. As the sulfur compounds at the same time are kept at almost the same level as for the fresh leek slices, there is no doubt that the nitrogen packaging is maintaining the aroma profile of the fresh leek much better than the atmospheric air packaging.

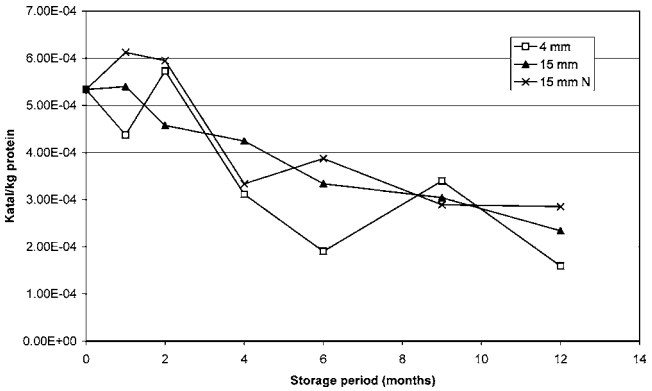

Figure 5. Specific activity of ADH analyzed in leek slices during frozen storage. See **Table 3** for specification of statistical differences within one treatment.

The production of ketones, alcohols, and furans are not affected by the slice thickness neither in the fresh leek slices nor after the frozen storage, as there are no statistical differences between 4- and 15-mm slices at each point of analysis. The effect of frozen storage is clear though, as all of the compounds except 2-pentyl furan increase during storage. Nitrogen packaging reduces this development during the storage, as these compounds are found in significantly lower concentrations than in the 15-mm slices.

Figures 3−**5** show the enzymatic activity of LOX, HPL, and ADH. LOX activity (**Figure 3**) decreases during the first two months, and after that, the activity is constant for 4- and 15-mm slices. There is no statistical difference between the two slice thicknesses, which indicates that the ability of LOX in the frozen tissue to gain activity when thawed is not influenced by slice thickness. After the drop in activity, 15-mm N increases slightly again, and apart from one inexplicable result from 15-mm N after 9 months of storage, which is higher than at the start, the activity ends up being significantly higher than the slices packed in atmospheric air. It is not obvious why the LOX activity is showing a maximum at 9 months of storage under nitrogen when tested under assay conditions. However, as the LOX reaction involves oxygen as the second substrate; a stabilizing influence of longer storage under nitrogen on the activity of LOX could possibly be anticipated.

HPL activity (**Figure 4**) is decreased during storage, reaching a minimum after 4−9 months of storage. HPL is not affected by nitrogen packaging, but slice thickness results in significantly higher activity after 12 months of frozen storage. ADH activity (**Figure 5**) decreases all through storage but is not significantly influenced by slice thickness or atmosphere after 12 months of frozen storage.

Most of the sulfur compounds decrease effectually during storage when packaged in atmospheric air, but all sulfur compounds are still present after 12 months of frozen storage. The 4-mm slices produce significantly most of nearly all of the sulfur compounds both in the fresh leek slices and after storage, but these slices also generate the largest concentration of aldehydes, which appears as off-flavor. Nitrogen packaging has a great influence on the keeping of aroma compounds of leek slices during frozen storage, as the 15-mm N almost maintains the aroma profile of the fresh leek slices. The content of sulfur compounds in the 15-mm N after 12 months of frozen storage is kept at almost the same level as the fresh leek slices, and the generation of aldehydes, ketones, and alcohols are reduced significantly compared to 4- and 15-mm stored for 12 months. Slice thickness does not influence the activity of LOX and ADH, but 4-mm slices result in higher activity of HPL than 15-mm slices do. Nitrogen packaging results in higher activity of LOX after 12 months of frozen storage compared to atmospheric air, while HPL and ADH are unaffected.

ABBREVIATIONS USED

LOX, lipoxygenase; HPL, hydroperoxide lyase; ADH, alcohol dehydrogenase; 15 mm N, 15 mm leek slices packed in nitrogen; 12M, 12 months of frozen storage

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Received for review August 22, 2003. Revised manuscript received December 5, 2003. Accepted December 6, 2003.

JF0306183