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Impact of Blanching and Packaging Atmosphere on the Formation of Aroma Compounds during Long-Term Frozen Storage of Leek (*Allium ampeloprasum* Var. *Bulga*) Slices

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The content of aroma compounds and the catalytic activity of lipoxygenase (LOX), alliinase, hydroperoxide lyase (HPL), and alcohol dehydrogenase (ADH) were analyzed in unblanched and blanched 15-mm leek slices packed in atmospheric air (21% O_2) or 100% nitrogen (0% O_2) three times during 12 months of frozen storage (12 M). The total amount of sulfur compounds and the total amount of aldehydes were greatly influenced by storage time, atmosphere, and blanching [concentration of sulfur compounds in fresh unblanched (UNB) slices = 1.35 mg/L, fresh blanched (B) slices = 1.09 mg/L, UNB 21% O_2 12 M = 0.656 mg/L, UNB 0% O_2 12 M = 2.11 mg/L, B 21% O_2 12 M = 1.14 mg/L, B 0% O_2 12 M = 1.59 mg/L]. B 0% O_2 was closest to the original ratio between sulfur compounds and aldehydes after 12 months. The activities of HPL and alliinase were totally lost after 12 months, and ADH showed minimal activity, whereas LOX (UNB 0% O_2) showed ~25% of the original activity. LOX was the most and HPL the least heat labile enzyme investigated.

KEYWORDS: Leek; flavor; frozen storage; lipoxygenase; hydroperoxide lyase; alcohol dehydrogenase; alliinase; packaging atmosphere; blanching

INTRODUCTION

The chemical composition of *Allium* plants is characterized by a high amount of organic bound sulfur compared to most other plants (*1*). This determines the aroma of freshly cut leek and other *Allium* species, which mainly consist of numerous sulfur-containing volatile compounds. These compounds originate from the alliinase (EC 4.4.1.4)-catalyzed decomposition of the odorless nonvolatile precursors *S*-alk(en)yl cysteine sulfoxides to sulfenic acids (*2*, *3*). Sulfenic acids are highly reactive (*4*) and will quickly combine to form thiosulfinates. Thiosulfinates are responsible for the odor of freshly cut leeks (*4*, *5*), but as they are relatively unstable (*2*, *6*) they will rearrange to form polysulfides and thiosulfonates. Thiosulfonates are transformed to the corresponding monosulfide, and polysulfides can rearrange to mono- and trisulfides, so the final products of the reactions will be a mixture of mono- and polysulfides with all of the possible combinations of the *S*-alk(en)yl radicals of common occurrence. The existence of these radicals is dependent on the species; in leek the presence of methyl, ethyl, propyl, 1-propenyl, and 2-propenyl radicals has been reported (*4*, *6*, *7*).

The lipoxygenase (LOX) pathway is another contributor to the formation of aroma in fresh and especially in stored leek slices. LOX (EC 1.13.11.12) catalyzes the peroxidation of polyunsaturated fatty acids with a (*Z*,*Z*)-1,4-pentadiene moiety (*8*, *9*) under the consumption of dioxygen. The polyunsaturated fatty acids are present in cell membranes and have to be liberated before initiation of the reactions (*10*). This happens during the cutting and cleaning process of the vegetable or by natural loss of cell structure during senescence. Volatile aldehydes are produced by the action of hydroperoxide lyase (HPL) upon the formed hydroperoxides (*8*, *11*). 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 (*11*). These fatty acid-derived compounds can also generate by autoxidation during the storage period (*12*), and both pathways will result in accumulation of the compounds in the tissue and in the headspace of retail packages.

The formation of aldehydes and alcohols contributes to the aroma of fresh leeks when present in minor concentration but will probably be perceived as off-flavors when present in larger amounts (*13*, *14*). This is not very pronounced in the fresh leek because of the pungency of thiosulfinates and thiopropanal-*S*-oxide (*6*). However, previous investigations of unblanched frozen leek slices (*13*, *15*, *16*) have shown that the storage period has a great influence on the aroma profile of the leek slices as the concentration of sulfur compounds decreases and that of

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aldehydes (saturated and nonsaturated) increases effectively during the storage.

Production of frozen vegetable often includes a blanching step mainly to destroy the catalytic activity of enzymes and prevent off-flavor formation. In addition, microorganisms are killed and air is expelled from the tissue, which can reduce autoxidation during storage. Usually losses of flavor, vitamins, and texture are consequences of the blanching process and the subsequent cooling though (*17*, *18*), and especially for leek slices the loss of texture is detrimental to the quality.

A previous investigation (*15*) on nitrogen packaging of frozen unblanched leek slices has shown that after 1 year of frozen storage the development of off-flavor was reduced and the content of sulfur compounds was kept almost at the level of fresh leek slices, when the slices were stored in 100% nitrogen. Consequently, both blanching and nitrogen packaging could retard oxidation during frozen storage and thus result in less accumulation of off-flavor in the leek slices, but as the nitrogen packaging does not influence texture, it is valuable to compare the two methods.

The objective of the present study was to investigate the impact of blanching and packaging atmosphere on aroma formation and enzyme activity in frozen leek slices stored for 12 months.

MATERIALS AND METHODS

Plant Material. Leeks (*Allium ampeloprasum* var. *Bulga*) were harvested fully matured at Funen, Denmark, in October 2001 and used for the experiment immediately after.

Chemicals. The reference compounds (purity ≥ 97%) 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-nonenal, 2-methyl-(*E*)-2-butenal, 2-methyl-(*E*)-2-pentenal, (*E*,*E*)-2,4-heptadienal, (*E*,*E*)-2,4-decadienal, 2-heptanone, 2-octanone, 3-octanone, 1-propanol, 1-pentanol, 1-hexanol, 1-octen-3-ol, propanoic acid, hexanoic acid, and 2-pentylfuran were bought commercially from Sigma-Aldrich, Copenhagen, Denmark. (*E*,*Z*)-2,4-Heptadienal occurred as an impurity in (*E*,*E*)-2,4-heptadienal. NADH and lactate dehydrogenase (LDH) were bought commercially from Roche Diagnostics Scandinavia AB, Hvidovre, Denmark. All other chemicals, except for *S*-methyl-L-cysteine sulfoxide and 9-(*S*)-hydroperoxy-(10*E*,12*Z*)-octadecadienoic acid, were of analytical grade and were bought commercially from Sigma-Aldrich.

Synthesis of *S*-Methyl-L-cysteine Sulfoxide. The procedure was modified after that of ref *19*. Fifty milliliters of *S*-methyl-L-cysteine (0.6 M in deionized water) and 3.80 mL of hydrogen peroxide (30%) were mixed for 24 h at 24 °C with agitation (100 rpm). The generated cysteine sulfoxide was precipitated by 50 mL of cold (3 °C) ethanol for 12 h at 3 °C. Separation of the precipitation product was done through a paper filter. The precipitate was dried and afterward dissolved in 5 mL of 0.2 M phosphate buffer (pH 6.0) and kept at 3 °C in the dark until analysis.

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 (*20*), 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 filtered through a paper filter. A 2-mL aliquot of the liquid was applied to a DEAD-Cellulose C545 column (anion exchanger) (Sigma-Aldrich) 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. *Unblanched Samples.* 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 reached −20 °C.

Blanched Samples. The white part of the leek stem was cut into 15-mm slices, and immediately after portions of 500-g slices were immersed in a pot with a lid containing 3 L of boiling water for 225 s. After the blanching, the leek slices were dripped off for 15 s, cooled in crushed ice for 15 min, and frozen in a blast freezer at −20 °C for 10 min, so that the center temperature reached −20 °C.

Both unblanched and blanched frozen leek slices were packed in airtight glass jars with atmospheric air or 100% nitrogen 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 unblanched and blanched slices were analyzed at harvest time, and subsequently two individual samples of unblanched and blanched slices packed in atmospheric air and in 100% nitrogen were analyzed after 6 and 12 months of frozen storage. Each sample was analyzed for composition of atmosphere in the glass jars, catalytic activity of LOX, HPL, ADH, and alliinase, 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 atmosphere analyzer from Systech Instruments Ltd., Oxon, U.K.

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. The enzyme extract was desalted by size exclusion chromatography through a PD-10 column (Amersham Biosciences, Uppsala, Sweden) with Sephadex G-25 medium prior to analysis of HPL, ADH, and alliinase.

LOX Assay. LOX was assayed in a continuous assay by measuring consumption of initial dioxygen by a 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 the addition of sodium dithionite (0% dissolved oxygen). To determine LOX activity, 27.2 mL of air-saturated tempered phosphate buffer (0.2 M, pH 6.0) and 3.9 mL of enzyme extract were 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 by 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 kilogram of protein, and katals were defined as moles of O_2 consumed 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 of O_2/L (*21*). To determine the activity, the dioxygen level (millimoles of O_2) was plotted against time, and the steepest slope (millimoles of O_2/s) on the curve was found using continuous linear regression over 20 s at a time.

HPL Assay. HPL was assayed in a continuous coupled assay modified after that of ref *22*. 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 NADH (2.2 mM in deionized water), 200 μL of ADH [150 units in 100 μL potassium phosphate buffer (50 mM, pH 7.0)], and 300 μL of enzyme extract were transferred to a thermostatic (30 °C) 1-cm cuvette with a magnetic stirrer, and the measuring was started. After 60 s, 400 μL of 9-HPODE was added. The reaction was monitored over 600 s by measuring every second on an HP 8453A UV−vis spectrophotometer.

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 kilogram of protein, and katals were defined as moles of hydroperoxide consumed per second. 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 1 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 (*22*).

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

Borate buffer (1.4 mL, 0.05 M, pH 9.0), 300 μL of NADH (2.2 mM in deionized water), and 1000 μL of enzyme extract were transferred to a thermostatic (30 °C) 1-cm cuvette with a magnetic stirrer, and the measuring was started. After 60 s, 300 μL of hexanal (100 ppm in deionized water) was added. The reaction was monitored over 600 s by measuring every second on am HP 8453A UV−vis spectrophotometer.

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

Calculation of ADH Activity. ADH activity was calculated as katals per kilogram of protein, and katals were defined as moles of hexanal consumed per second. 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.

Alliinase Assay. Alliinase was assayed in a continuous coupled assay. LDH converts pyruvate, one of the products of the alliinases reaction on *S*-methyl-L-cysteine sulfoxide, under the oxidation of NADH, which can be monitored spectrophotometrically at 340 nm.

Phosphate buffer (2.1 mL, 0.2 M, pH 6.0), 300 μL of NADH (2.2 mM in deionized water), 10 μL of pyridoxal-5′-phosphate (6 mM in deionized water), 50 μL of LDH [900 units in 1 mL of 50% glycerol (v/v), pH 6.5], and 600 μL of enzyme extract were transferred to a thermostatic (30 °C) 1-cm cuvette with a magnetic stirrer, and the measuring was started. After 60 s, 40 μL of *S*-methyl-L-cysteine sulfoxide (0.18 M in 0.2 M phosphate buffer, pH 6.0) was added. The reaction was monitored over 600 s by measuring every second on an HP 8453A UV−vis spectrophotometer.

Blank samples were run on buffer, NADH, pyridoxal-5′-phosphate, and LDH added substrate or enzyme extract.

Calculation of Alliinase Activity. Alliinase activity was calculated as katals per kilogram of protein, and katals were defined as moles of *S*-methyl-L-cysteine sulfoxide consumed per second. Moles of *S*-methyl-L-cysteine sulfoxide were calculated on the basis of NADH under the assumption that each mole of *S*-methyl-L-cysteine sulfoxide metabolized produced 1 mole of pyruvate. Pyruvate is converted by lactate dehydrogenase by the consumption of NADH 1:1. Other conditions were as 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 Netherlands) 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, U.K.). The desorption temperature of the trap to the cold trap (contains 30 mg of Tenax GR, temperature = 5 °C) was 250 °C for 15 min with a helium flow of 60 mL/min. The 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, Folsom, CA (30 m × 0.25 mm i.d. × 0.25-μm film thickness); carrier gas, helium; start flow, 1 mL/min; split ratio, none; column pressure, 48 kPa (constant); oven program, 45 °C for 10 min, raised at 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-nonenal, 2-methyl-(*E*)-2-butenal, 2-methyl-(*E*)-2-pentenal, (*E*,*E*)-2,4-heptadienal, (*E*,*E*)-2,4-decadienal, 2-heptanone, 2-octanone, 3-octanone, 1-propanol, 1-pentanol, 1-hexanol, 1-octen-3-ol, propanoic acid, hexanoic acid, and 2-pentylfuran.

Quantitation. 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 was performed on 400 mL of the solutions with added 4 mL of internal standard (50 ppm 4-methyl-1-pentanol in tap water) 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, propyl butyl disulfide, and propyl pentyl disulfide after dipropyl disulfide; diisopropyl trisulfide and 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 and 3,5-octadiene-2-one after 3-octanone; and 2,5-dimethylfuran after 2-pentylfuran.

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 ref *23*.

Statistical Analysis. Analysis of variance was carried out on the catalytic activities of LOX, HPL, ADH, and alliinase 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, blanching, and packaging atmosphere was tested. Sample means were compared by Duncan's multiple-range test. A significance level of 5% was applied.

RESULTS AND DISCUSSION

The unblanched slices packed in atmospheric air (UNB 21% O_2) modified the atmosphere in the glass jars to 14.5% O_2 and 5.6% CO_2 after 12 months of frozen storage (**Table 1**). This indication of respiration in the leek slices during frozen storage was also found in a previous study (*15*), which also revealed that a greater extent of shredding gave less modification of the atmosphere. The blanched slices packed in atmospheric air (B 21% O_2) did not modify the atmosphere. The nitrogen packaging, both unblanched (UNB 0% O_2) and blanched (B 0% O_2), kept the atmosphere in the jars constant at 98−99% N_2 except for the UNB 0% O_2 after 6 months of storage, which for some reason held 7.1% O_2, 1.0% CO_2, and 91.9% N_2. The atmosphere composition after 12 months showed that minor respiration also

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

		storage period	
		6 months	12 months
UNB[a] 21% O_2[b]	O_2	17.7	14.5
	CO_2	3.5	5.6
	N_2	78.8	79.9
B[c] 21% O_2	O_2	20.6	20.0
	CO_2	0.3	0.6
	N_2	79.1	79.4
UNB 0% O_2[d]	O_2	7.1	0.5
	CO_2	1.0	1.5
	N_2	91.9	98.0
B 0% O_2	O_2	2.1	1.1
	CO_2	0.0	0.0
	N_2	97.9	98.9

[a] Unblanched slices. [b] Initial atmosphere: atmospheric air. [c] Blanched slices. [d] Initial atmosphere: 100% nitrogen.

Table 2. Total[a] of Sulfur Compounds and Aldehydes Found in Fresh Leek (0 M) and in Leek Stored Frozen for 12 Months (12 M)

compound	sulfur	aldehydes[b]
UNB[c] 0 M	1.35 ± 0.451	0.681 ± 0.206
UNB 21% O_2[d] 12 M	0.656 ± 0.181	7.86 ± 2.25
UNB 0% O_2[e] 12 M	2.11 ± 0.216	4.63 ± 0.774
B[f] 0 M	1.09 ± 0.228	0.477 ± 0.105
B 21% O_2 12 M	1.14 ± 0.090	2.13 ± 0.404
B 0% O_2 12 M	1.59 ± 0.129	0.647 ± 0.042

[a] All values are in mg/L ± standard deviation. [b] Not including propanal, 2-methyl-2-butenal, and 2-methyl-2-pentenal; see text. [c] Unblanched slices. [d] Atmospheric air. [e] 100% nitrogen. [f] Blanched slices.

occurred in the UNB 0% O_2 as these samples contained 0.5% O_2 and 1.5% CO_2, whereas the B 0% O_2 held 1.1% O_2 and 0.0% CO_2.

UNB 21% O_2 samples produced a total amount of sulfur compounds of 1.35 mg/L as fresh, which decreased significantly to 0.656 mg/L after 12 months of frozen storage (**Table 2**). This is consistent with previously reported results (*13*, *15*). Five of the sulfur compounds [ethyl 1-methylethyl disulfide, propyl butyl disulfide, diisopropyl trisulfide, and both isomers of propyl propenyl trisulfide] actually increased significantly during storage, and for at least three of them, this was substantial (**Table 3**). Five other compounds (methyl 2-propenyl disulfide, one isomer of propyl propenyl disulfide, propyl pentyl disulfide, and 2,5-dimethylthiophene) were present in constant amounts during the storage period, whereas the amount of the remaining nine sulfur compounds declined significantly through the period. No thiosulfinates were detected in the freshly cut leek slices; this is explained by the isolation method and the GC-MS method that most likely decomposes these compounds, if they are present (*4*, *13*).

The blanching process reduced the total production of sulfur compounds in the fresh samples to 1.09 mg/L (**Table 2**), but still six compounds [ethyl 1-methylethyl disulfide, dipropyl disulfide, one isomer of propyl propenyl disulfide, propyl butyl disulfide, propyl pentyl disulfide, and 3,4- or 2,4-dimethylthiophene] were produced in statistically equal amounts and 1-propanethiol and 2,5-dimethylthiophene were increased (**Table 3**). These two compounds have by other authors been shown to increase during heat treatment of onions (*1*, *24*). The blanching process stabilized the decomposition of sulfur compounds during frozen storage as the B 21% O_2 samples stored for 12 months contained statistically the same amount of sulfur compounds (1.14 mg/L) (**Table 2**) as the fresh blanched samples and higher amounts than the UNB 21% O_2 12 M samples.

About half of the aldehydes were not detected at all in the fresh unblanched slices (**Table 3**), but they developed during frozen storage and the ones already present in the fresh unblanched slices also increased during storage. Exceptions were propanal, (*E*)-2-hexenal, (*E*,*Z*)-2,4-heptadienal, and (*E*,*E*)-2,4-heptadienal, which were present in statistically equal amounts before and after frozen storage. The total amount of aldehydes [not including propanal, 2-methyl-(*E*)-2-butenal, and 2-methyl-(*E*)-2-pentenal, which are breakdown products from the lachrymatory factor thiopropanal-*S*-oxide (*25*)] increased from 0.681 to 7.86 mg/L (**Table 2**) in the UNB 21% O_2 12 M, and the majority of this effectual increase was due to pentanal, hexanal, and (*E*)-2-butenal. Previous investigations (*13*, *15*) showed an equivalent increase of aldehydes. In the blanched samples this development was not as noticeable as the total amount of aldehydes was 0.477 mg/L in the fresh samples and 2.13 mg/L in the B 21% O_2 12 M samples, but still the difference was significant. Six compounds [(*E*)-2-butenal, (*E*)-2-nonenal, (*E*,*Z*)-2,4-heptadienal, (*E*,*E*)-2,4-heptadienal, (*E*,*E*)-2,4-decadienal, and 2-ethyl-(*E*)-2-butenal] did not develop at all in the B 21% O_2 during the 12 months of frozen storage and propanal decreased (**Table 3**).

There was a minor difference between the fresh unblanched and blanched samples as six of the aldehydes [butanal, hexanal, heptanal, (*E*)-2-hexenal, (*E*)-2-heptenal, and 2-methyl-(*E*)-2-pentenal] decreased after blanching and pentanal and decanal increased. After 12 months of frozen storage, this difference was more pronounced as the total amount of aldehydes was 7.86 mg/L in the UNB 21% O_2 samples and 2.13 mg/L in the B21% O_2 samples (**Table 2**).

The alcohols, ketones, furans, and acids were not formed in the fresh samples but developed during frozen storage in the unblanched samples (**Table 3**). In the blanched samples this development was not seen, as many of the compounds were not formed at all. An exception was 1-octen-3-ol, which developed from 0.0 mg/L to 0.222 mg/L in the B21% O_2 12 M.

Whereas UNB 21% O_2 showed a decline of >50% of the total content of sulfur compounds during frozen storage, the nitrogen packaging had a very positivel effect on the keeping of sulfur compounds as the total content of sulfur compounds after 12 months of frozen storage under nitrogen was 2.11 mg/L compared to the 1.35 mg/L in the fresh slices (**Table 2**), and 12 of the compounds [methyl propyl disulfide, ethyl 1-methylethyl disulfide, dipropyl disulfide, one isomer of propyl propenyl disulfide, propyl 2-propenyl disulfide, propyl butyl disulfide, propyl pentyl disulfide, diisopropyl trisulfide, both isomers of propyl propenyl trisulfide, 1-propanethiol, and 2,5-dimethylthiophene] actually increased significantly during storage (**Table 4**). This signifies that the UNB 0% O_2 samples stored for 12 months had the statistically highest total concentration of sulfur compounds of all. An increase of sulfur compounds altogether was also seen in the blanched samples stored under nitrogen, but 10 of the sulfur compounds [methyl propyl disulfide, methyl 2-propenyl disulfide, dipropyl disulfide, one isomer of propyl propenyl disulfide, propyl butyl disulfide, propyl pentyl disulfide, dimethyl trisulfide, diisopropyl trisulfide, one isomer of propyl propenyl trisulfide, and 2,5-dimethylthiophene] were present in statistically equal amounts before

Table 3. Aroma Compounds[a] Found in Fresh Leek (0 M) and in Leek Stored Frozen for 12 Months (12 M) in Atmospheric Air

compound	RTI[b]	UNB[c] 0 M	UNB 21% O_2 12 M	B[d] 0 M	B 21% O_2 12 M
sulfur compounds					
1-propanethiol	857	0.0432 ± 0.0174	0.0269 ± 0.00404	0.281 ± 0.0672	0.0514 ± 0.00887
dimethyl disulfide	1086	0.0363 ± 0.0166	0.00355 ± 0.000784	0.00706 ± 0.00215	0.00714 ± 0.000547
methyl propyl disulfide	1242	0.0917 ± 0.0466	0.0636 ± 0.0258	0.0168 ± 0.00658	0.0247 ± 0.00248
methyl 2-propenyl disulfide[e]	1296	0.000123 ± 0.0000728	0.0000599 ± 0.0000132	0.0000284 ± 0.0000354	0.00 ± 0
methyl propenyl disulfide[e,f]	1263	0.0508 ± 0.0166	0.00266 ± 0.000642	0.0318 ± 0.00596	0.0339 ± 0.00410
methyl propenyl disulfide[e,f]	1292	0.166 ± 0.0586	0.00884 ± 0.00304	0.0570 ± 0.0120	0.0899 ± 0.00905
ethyl 1-methylethyl disulfide[e]	1319	0.000314 ± 0.000170	0.00227 ± 0.000718	0.000166 ± 0.000101	0.00 ± 0
dipropyl disulfide	1387	0.62 ± 0.180	0.387 ± 0.107	0.461 ± 0.218	0.500 ± 0.0609
propyl 2-propenyl disulfide[e]	1436	0.0000660 ± 0.0000404	0.0000739 ± 0.0000256	0.00 ± 0	0.0000490 ± 0.0000117
propyl propenyl disulfide[e,f]	1422	0.00754 ± 0.00271	0.00633 ± 0.00231	0.00856 ± 0.00146	0.00940 ± 0.00103
propyl propenyl disulfide[e,f]	1438	0.0858 ± 0.0378	0.0271 ± 0.0123	0.0306 ± 0.00424	0.0435 ± 0.00390
propyl butyl disulfide[e]	1493	0.00 ± 0	0.0000117 ± 0.00000256	0.00 ± 0	0.00 ± 0
propyl pentyl disulfide[e]	1568	0.00 ± 0	0.0000395 ± 0.0000446	0.00 ± 0	0.0000115 ± 0.00000205
dimethyl trisulfide	1376	0.0383 ± 0.0157	0.00240 ± 0.000566	0.00182 ± 0.000525	0.00240 ± 0.000566
diisopropyl trisulfide[e]	1656	0.0212 ± 0.00540	0.0695 ± 0.0230	0.00888 ± 0.00409	0.0151 ± 0.00192
propyl propenyl trisulfide[e,f]	1781	0.000984 ± 0.000161	0.00161 ± 0.000602	0.000328 ± 0.0000729	0.000486 ± 0.000113
propyl propenyl trisulfide[e,f]	1770	0.00120 ± 0.000188	0.00180 ± 0.000619	0.000370 ± 0.0000974	0.000583 ± 0.000141
2,5-dimethylthiophene	1190	0.00498 ± 0.000679	0.00464 ± 0.00440	0.0109 ± 0.00238	0.0100 ± 0.00125
3,4- or 2,4-dimethylthiophene[e]	1253	0.185 ± 0.0706	0.0501 ± 0.0121	0.172 ± 0.0270	0.348 ± 0.0352
aldehydes					
propanal	762	2.86 ± 0.336	2.16 ± 0.712	2.42 ± 1.66	0.705 ± 0.112
butanal	867	0.00612 ± 0.000828	0.0804 ± 0.00601	0.00 ± 0	0.0245 ± 0.00155
pentanal	984	0.0468 ± 0.0121	1.55 ± 0.212	0.375 ± 0.102	0.799 ± 0.0514
hexanal	1111	0.313 ± 0.0878	4.74 ± 1.42	0.0267 ± 0.00320	0.784 ± 0.154
heptanal	1197	0.0262 ± 0.00323	0.125 ± 0.0322	0.0207 ± 0.00216	0.0753 ± 0.00964
octanal	1299	0.00 ± 0	0.0132 ± 0.00315	0.00 ± 0	0.0161 ± 0.00338
nonanal	1398	0.00 ± 0	0.0114 ± 0.00503	0.00 ± 0	0.0289 ± 0.00361
decanal	1510	0.00 ± 0	0.000885 ± 0.000474	0.000847 ± 0.000358	0.00163 ± 0.000388
(*E*)-2-butenal	1046	0.00 ± 0	0.652 ± 0.0519	0.00 ± 0	0.00 ± 0
(*E*)-2-pentenal	1147	0.00 ± 0	0.109 ± 0.0597	0.00 ± 0	0.0297 ± 0.0103
(*E*)-2-hexenal	1230	0.167 ± 0.108	0.0862 ± 0.0170	0.00728 ± 0.00516	0.0130 ± 0.00277
(*E*)-2-heptenal	1334	0.0545 ± 0.00364	0.140 ± 0.0835	0.0464 ± 0.00275	0.357 ± 0.194
(*E*)-2-nonenal	1532	0.00 ± 0	0.00317 ± 0.00210	0.00 ± 0	0.00 ± 0
2-methyl-(*E*)-2-butenal	1113	0.00 ± 0	3.01 ± 0.257	0.00 ± 0	0.0900 ± 0.00600
2-ethyl-(*E*)-2-butenal[e]	1166	0.00 ± 0	0.0825 ± 0.0100	0.00 ± 0	0.00 ± 0
2-methyl-(*E*)-2-pentenal	1176	2.55 ± 0.285	0.101 ± 0.0252	0.0289 ± 0.00275	0.210 ± 0.0325
(*E*,*Z*)-2,4-heptadienal[e]	1464	0.0414 ± 0.0109	0.0223 ± 0.0197	0.00 ± 0	0.00 ± 0
(*E*,*E*)-2,4-heptadienal	1490	0.0248 ± 0.00193	0.0148 ± 0.0136	0.00 ± 0	0.00 ± 0
(*E*,*E*)-2,4-decadienal	1789	0.00 ± 0	0.226 ± 0.485	0.00 ± 0	0.00 ± 0
ketones					
2-heptanone	1180	0.00 ± 0	0.00681 ± 0.000633	0.00 ± 0	0.00 ± 0
2-octanone	1297	0.00 ± 0	0.0199 ± 0.00176	0.00 ± 0	0.00 ± 0
3-octanone	1272	0.00 ± 0	0.0139 ± 0.00194	0.00 ± 0	0.00 ± 0
3-octen-2-one[e]	1345	0.00 ± 0	0.00675 ± 0.000420	0.00 ± 0	0.00 ± 0
3,5-octadien-2-one[e]	1516	0.00 ± 0	0.00604 ± 0.00180	0.00 ± 0	0.00 ± 0
alcohols					
1-propanol	1049	0.563 ± 0.163	0.00 ± 0	0.324 ± 0.169	0.483 ± 0.0409
1-pentanol	1274	0.00 ± 0	0.471 ± 0.135	0.00 ± 0	0.00 ± 0
1-hexanol	1371	0.00 ± 0	0.153 ± 0.0361	0.00 ± 0	0.00 ± 0
1-octen-3-ol	1458	0.00 ± 0	0.0642 ± 0.0132	0.00 ± 0	0.222 ± 0.107
acids					
propanoic acid	1564	0.0406 ± 0.0143	0.123 ± 0.0231	0.0286 ± 0.00737	0.0161 ± 0.00407
hexanoic acid	1816	0.0591 ± 0.0302	1.89 ± 1.01	0.0289 ± 0.00738	0.0633 ± 0.0242
furans					
2,5-dimethylfuran[e]	958	0.00498 ± 0.000679	0.0228 ± 0.00647	0.00 ± 0	0.00 ± 0
2-pentylfuran	1249	0.0157 ± 0.00473	0.0335 ± 0.0327	0.00445 ± 0.000495	0.0190 ± 0.00366

[a] All values except RTI are in mg/L ± standard deviation. [b] Retention time index. [c] Unblanched slices. [d] Blanched slices. [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.

and after 12 months of frozen storage under nitrogen. The observed increase was mostly due to the development of 3,4- or 2,4-dimethylthiophene, which increased almost 5 times during storage.

The development of aldehydes was also significantly influenced by storage under nitrogen as the total amount in the UNB 0% O_2 samples after frozen storage was 4.63 mg/L compared to 7.86 mg/L in UNB 21% O_2 (**Table 2**). When the two blanched samples (B 21% O_2 and B 0% O_2) were compared with UNB 0% O_2 (**Tables 3** and **4**) after 12 months of frozen storage, most of the aldehydes were present in statistically higher amounts in the UNB 0% O_2. The crucial difference between the three treatments, though, was the development of hexanal, which accounts for almost all of the difference in the total content of aldehydes. The formation of this compound should be inhibited by low oxygen, as it is a product of the lipoxygenase pathway and autoxidation, and this was also demonstrated by (*15*). Blanching prevented the formation of this compound,

Table 4. Aroma Compounds[a] Found in Leek Stored Frozen for 12 Months (12 M) in 100% Nitrogen

compound	RTI[b]	UNB[c] 0% O_2 12 M	B[d] 0% O_2 12 M
sulfur compounds			
1-propanethiol	857	0.0782 ± 0.0115	0.0964 ± 0.0380
dimethyl disulfide	1086	0.00349 ± 0.00128	0.00131 ± 0.000174
methyl propyl disulfide	1242	0.161 ± 0.0251	0.0161 ± 0.00309
methyl 2-propenyl disulfide[e]	1296	0.0000942 ± 0.0000154	0.00 ± 0
methyl propenyl disulfide[e,f]	1263	0.00975 ± 0.0154	0.0249 ± 0.00219
methyl propenyl disulfide[e,f]	1292	0.0285 ± 0.00503	0.0806 ± 0.00225
ethyl 1-methylethyl disulfide[e]	1319	0.00655 ± 0.000979	0.00 ± 0
dipropyl disulfide	1387	1.03 ± 0.131	0.517 ± 0.0859
propyl 2-propenyl disulfide[e]	1436	0.000196 ± 0.0000491	0.0000399 ± 0.00000998
propyl propenyl disulfide[e,f]	1422	0.0163 ± 0.00305	0.00946 ± 0.000891
propyl propenyl disulfide[e,f]	1438	0.0477 ± 0.00899	0.0450 ± 0.00235
propyl butyl disulfide[e]	1493	0.0000271 ± 0.00000246	0.00 ± 0
propyl pentyl disulfide[e]	1568	0.000119 ± 0.0000426	0.00 ± 0
dimethyl trisulfide	1376	0.00943 ± 0.00199	0.00170 ± 0.000262
diisopropyl trisulfide[e]	1656	0.622 ± 0.0444	0.0123 ± 0.00428
propyl propenyl trisulfide[e,f]	1781	0.00675 ± 0.00178	0.000718 ± 0.0000755
propyl propenyl trisulfide[e,f]	1770	0.00737 ± 0.00196	0.000931 ± 0.000122
2,5-dimethylthiophene	1190	0.00976 ± 0.00270	0.00924 ± 0.000835
3,4- or 2,4-dimethylthiophene[e]	1253	0.0790 ± 0.00986	0.770 ± 0.0567
aldehydes			
propanal	762	3.74 ± 0.775	0.405 ± 0.127
butanal	867	0.0287 ± 0.00303	0.00535 ± 0.00119
pentanal	984	0.575 ± 0.0572	0.394 ± 0.0333
hexanal	1111	2.95 ± 0.654	0.107 ± 0.0164
heptanal	1197	0.0724 ± 0.0138	0.0353 ± 0.00313
octanal	1299	0.00400 ± 0.000903	0.00679 ± 0.000688
nonanal	1398	0.0133 ± 0.00175	0.0358 ± 0.00646
decanal	1510	0.00135 ± 0.000703	0.00 ± 0
(*E*)-2-butenal	1046	0.166 ± 0.0192	0.00 ± 0
(*E*)-2-pentenal	1147	0.214 ± 0.0284	0.00 ± 0
(*E*)-2-hexenal	1230	0.234 ± 0.0298	0.00976 ± 0.00743
(*E*)-2-heptenal	1334	0.151 ± 0.0243	0.0532 ± 0.00551
(*E*)-2-nonenal	1532	0.00253 ± 0.00198	0.00 ± 0
2-methyl-(*E*)-2-butenal	1113	2.71 ± 0.629	0.0331 ± 0.00558
2-ethyl-(*E*)-2-butenal[e]	1166	0.0609 ± 0.0182	0.00 ± 0
2-methyl-(*E*)-2-pentenal	1176	0.358 ± 0.0485	0.146 ± 0.0222
(*E*,*Z*)-2,4-heptadienal[e]	1464	0.0615 ± 0.0113	0.00 ± 0
(*E*,*E*)-2,4-heptadienal	1490	0.0392 ± 0.00895	0.00 ± 0
(*E*,*E*)-2,4-decadienal	1789	0.0493 ± 0.0348	0.00 ± 0
ketones			
2-heptanone	1180	0.000137 ± 0.000191	0.00 ± 0
2-octanone	1297	0.0000723 ± 0.0000457	0.00 ± 0
3-octanone	1272	0.00285 ± 0.000308	0.0000417 ± 0.0000365
3-octen-2-one[e]	1345	0.000358 ± 0.0000717	0.00 ± 0
3,5-octadien-2-one[e]	1516	0.000850 ± 0.000351	0.00 ± 0
alcohols			
1-propanol	1049	5.04 ± 0.789	0.00 ± 0
1-pentanol	1274	0.390 ± 0.0582	0.00 ± 0
1-hexanol	1371	0.0312 ± 0.00264	0.00 ± 0
1-octen-3-ol	1458	0.0160 ± 0.00812	0.00 ± 0
acids			
propanoic acid	1564	0.296 ± 0.0629	0.0163 ± 0.0152
hexanoic acid	1816	1.656 ± 0.361	0.00 ± 0
furans			
2,5-dimethylfuran[e]	958	0.0000759 ± 0.0000512	0.00 ± 0
2-pentylfuran	1249	0.0472 ± 0.0137	0.0165 ± 0.00216

[a] All values except RTI are in mg/L ± standard deviation. [b] Retention time index. [c] Unblanched slices. [d] Blanched slices. [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.

which indicated that the large accumulation was due to enzymatic activity rather than autoxidation. The production of (*E*,*E*)-2,4-decadienal indicated the same as this compound was developed in only the unblanched samples stored in both atmospheres. This was also the case with (*E*)-2-butenal, (*E*)-2-nonenal, 2-ethyl-(*E*)-2-butenal, (*E*,*Z*)-2,4-heptadienal, and (*E*,*E*)-2,4-heptadienal.

Most of the ketones, alcohols, acids, and furans were increased in the UNB 0% O_2 during storage, whereas most of these compounds were not detected at all in the B 0% O_2 samples after 12 months and only 3-octanone and 2-pentylfuran increased in the B 0% O_2.

Investigations of LOX activity (**Figure 1a**) showed that activity decreased significantly in the two unblanched samples and that activity was influenced by the packaging atmosphere as UNB 0% O_2 gave significantly higher activity than UNB 21% O_2 at both 6 and 12 months of frozen storage. This stabilizing effect of nitrogen was also demonstrated by ref *15*. Blanching time was chosen so that there was no lipoxygenase activity left right after blanching, and the blanched samples

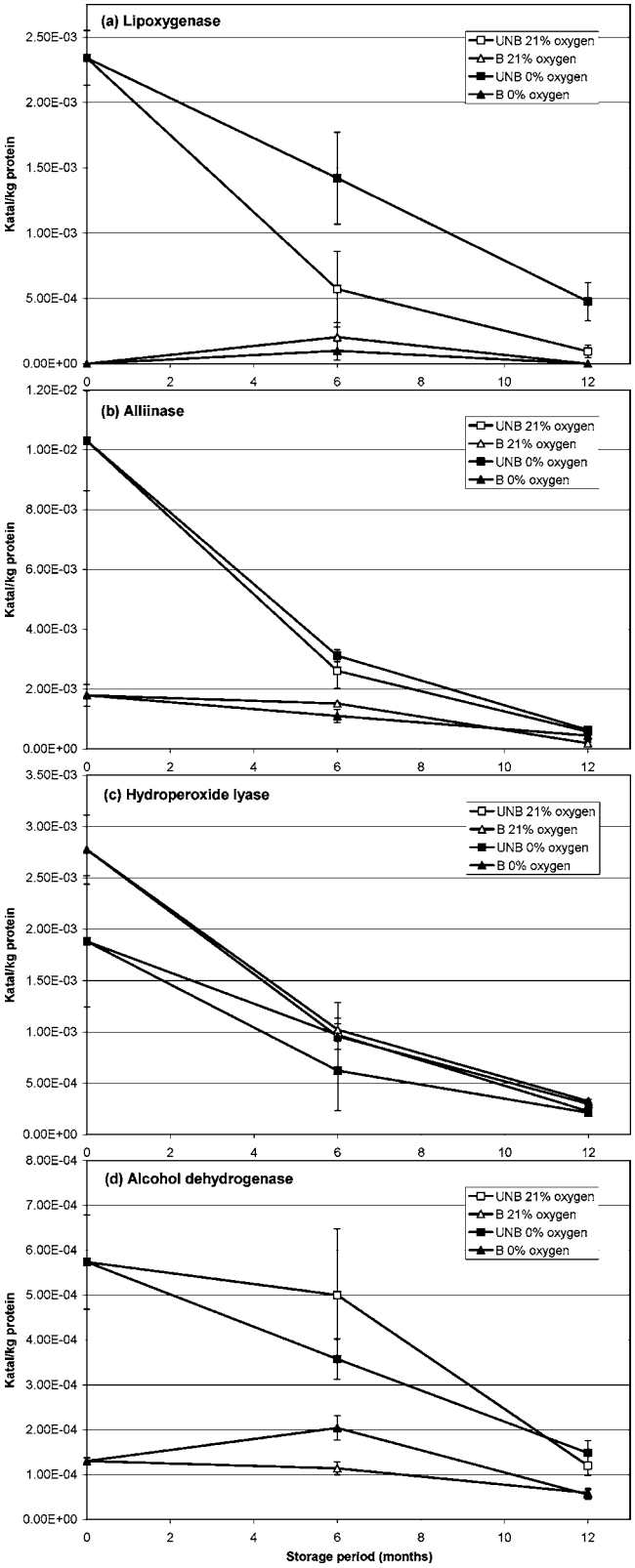

Figure 1. Specific activity of lipoxygenase (**a**), alliinase (**b**), hydroperoxide lyase (**c**), and alcohol dehydrogenase (**d**) analyzed in leek slices during frozen storage. UNB = unblanched. B = blanched. Packaging atmosphere is indicated as 21% oxygen = atmospheric air or 0% oxygen = 100% nitrogen. Vertical bars indicate standard deviation.

showed a constant low activity all through the frozen storage, which was not influenced by the packaging atmosphere. Unblanched leek slices lost activity during frozen storage, but the UNB 0% O_2 still had 25% of the initial activity left after 12 months, whereas the UNB 21% O_2 was statistically equal to the blanched samples.

Alliinase activity (**Figure 1b**) also demonstrated a decline in activity for the unblanched samples. In this case there was no difference between the two packaging atmospheres, which is probably a consequence of oxygen not being involved in the alliinase-catalyzed reaction. The two blanched samples showed very low activity right after the blanching process, but still a significant but minor loss of activity of alliinase occurred during frozen storage. This demonstrated that alliinase was not totally inactivated by the blanching procedure and that alliinase might be more heat resistant than LOX. After 6 months of frozen storage, the activity of unblanched samples was still significantly higher than that of the blanched samples, but at 12 months, the activities were equal. This demonstrates a lack of freezing stability of alliinase, which is in agreement with ref *26*, in which was reported the denaturation of alliinase during freezing. Freeman and Whenham (*27*) found intact activity of alliinase during 30 days of frozen storage of whole onion bulbs, and Wäfler et al. (*28*) found that the freezing stability of alliinase is dependent on the freezing technique more than the actual frozen storage, and only slow freezing led to loss of alliinase activity due to destruction of cell structure. Our results indicate that the storage time also influences the activity of alliinase.

Investigations of HPL activity (**Figure 1c**) evinced that HPL was not properly inactivated by the blanching process as the blanched fresh samples showed statistically the same activity as the unblanched fresh samples. All samples declined significantly during the frozen storage with no consequence on storage atmosphere. After 6 months of frozen storage, the activity had dropped to less than half of the initial activity, and after 12 months of frozen storage, practically no activity was detected. This demonstrated that the activity of HPL was sensitive to frozen storage over longer periods.

All treatments had a restrictive influence on ADH activity during the 12 months of frozen storage (**Figure 1d**). UNB 21% O_2 showed statistically the same activity after 6 months, though, but declined in the last 6 months of the storage period, whereas UNB 0% O_2 dropped significantly during the whole period. After 12 months of frozen storage, the two unblanched samples were equal but statistically higher than the two blanched samples. The blanched samples did show minor activity in the fresh samples, indicating that the blanching process was not sufficient to inactivate ADH. This activity remained almost constant during the storage period with a diminutive but significant decrease after 12 months. B 0% O_2 showed a minor increase after 6 months of frozen storage, which declined again.

In this study none of the four enzymes investigated was stable during frozen storage for longer periods, but whereas alliinase and HPL totally lost activity, LOX and ADH were still able to show some activity after 12 months of frozen storage in the unblanched samples. In the case of LOX this was influenced by the storage atmosphere as 0% O_2 gave the highest activity. LOX was, on the other hand, the most heat labile enzyme of the four investigated. LOX is the initiator of the lipoxygenase pathway, and that combined with the deterioration of texture with prolonged blanching time probably makes it sensible to choose the blanching time on the basis of LOX, which was also suggested by the authors of refs *18* and *29*. Whereas the reactions of LOX, HPL, and ADH mostly are unwanted, the action of alliinase during frozen storage would probably influence positively the headspace in retail packages of frozen leek slices.

The aroma analyses showed that storage in 100% nitrogen without blanching actually enhanced the content of sulfur compounds in the leek slices tremendously when compared to the fresh samples, but it also enhanced the production of aldehydes. In the case of aldehydes this could be a result of the better keeping of LOX activity found in the UNB 0% O_2, which will result in the formation of aldehydes during the crushing of leek slices prior to headspace analysis. This reflects the situation of thawing of the leek slices during cooking, and this activity is of course dependent on the condition of the enzymes at the actual time. In the case of sulfur compounds, alliinase activity should not be affected by the atmosphere, but nitrogen might have a preservative effect on the sulfur compounds or the precursors.

Comparison of the blanched and unblanched slices showed that the development of aldehydes was a result of both autoxidation and enzymatic activity. When stored in 21% O_2, the unblanched samples produced almost 4 times more aldehydes than the blanched samples, which indicates that mainly enzymatic activity is responsible for this production. This difference is both due to accumulation during the frozen storage and due to activity during the crushing of leek slices as explained above. The results show that it is possible to restrain autoxidation by packing without oxygen as the B 0% O_2 after 12 months had the same content of aldehydes as the fresh unblanched and blanched samples, whereas the concentration in B 21% O_2 after 12 months had tripled. When the ratio of total amount of sulfur compounds to total amount of aldehydes after 12 months of frozen storage was compared, the blanched samples stored in 100% nitrogen came closest to the ratio found in the fresh unblanched leek slices.

ABBREVIATIONS USED

UNB 21% O_2, unblanched slices packed in atmospheric air; B 21% O_2, blanched slices packed in atmospheric air; UNB 0% O_2, unblanched slices packed in 100% nitrogen; B 0% O_2, blanched slices packed in 100% nitrogen; 0 M, fresh leek; 12 M, 12 months of frozen storage; LOX, lipoxygenase; HPL, hydroperoxide lyase; ADH, alcohol dehydrogenase; LDH, lactate dehydrogenase; 9-HPODE, 9-(*S*)-hydroperoxy-(10*E*,12*Z*)-octadecadienoic acid.

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