

Model Studies on the Influence of Matrix Type and Storage Environment on the Stability of a Model Aroma Mixture during Storage

SEGOLENE LECLERCQ,^{*,†} GARY A. REINECCIUS,[†] AND CHRISTIAN MILO[‡]

Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Avenue, St. Paul, Minnesota 55108, and Nestlé Product Technology Center Orbe, CH1350 Orbe, Switzerland

The objective of this study was to investigate the effect of oxygen in the storage atmosphere on the degradation of model compounds when present in water or a medium chain triglyceride (MCT) matrix. A model aroma compound mixture was prepared in oil (MCT) or water, and it was then stored under either an ambient air or argon atmosphere containing respectively ca. 20% and <0.5% residual oxygen. Samples were analyzed by SPME-GC/MS to determine the relative stability over time of different classes of aroma compounds. The low-oxygen atmosphere appeared to have a significant protective effect on sulfur compounds, aldehydes, and ketones in oil but a detrimental influence on pyrroles. Data showed little influence of the atmosphere for these compounds in water. In addition, the type of matrix had a significant effect ($P < 0.05$) on the stability of aldehydic, ester, and pyrrole compounds. These compounds were more stable in MCT than in water.

KEYWORDS: Aroma stability; oxygen; storage; matrix effects; SPME

INTRODUCTION

Flavor deterioration in a food during storage has been extensively investigated in sensory studies, focusing mainly on the appearance of undesirable flavors. These off-flavors can result from various mechanisms involving lipid or terpene oxidation (e.g., in snack foods (1) or citrus beverages (2), respectively), nonenzymatic browning (e.g., in fruit juices (3)), enzymatic reactions (e.g., bitter notes in dairy products (2, 4)), or light induced reactions (e.g., beer staling (5)). It is, however, most probable that the staling of a food is not only due to the formation of off-flavors but also to the disappearance of desirable flavor. Nevertheless, there is limited literature on the degradation of desirable aroma compounds during storage. Williams et al. (6) found significant losses of several key components of roasted peanuts over time resulting in an increase in negative sensory attributes. Their study showed that staling can be due to combined effects of losses of desirable flavors and the appearance of undesirable ones.

The characteristics of the food matrix, such as presence of proteins or quantity of lipids in specific solid matrixes, have been shown to influence the stability of flavor compounds during storage (7, 8). However, there is little literature published at this time investigating the effect of storage conditions on the stability of flavorings in a liquid matrix other than the degradation of lemon flavor components in low-pH beverages (3).

The primary objective of the present study was to investigate the influence of the liquid carrier (oil vs water) on the stability of aroma compounds over time. One would anticipate that different degradation mechanisms would occur in a water solvent vs a lipid. A secondary objective was to determine the effect of oxygen level on aroma stability. Many foods are packaged under reduced oxygen to limit oxidative reactions leading to off-flavors. However, one must also recognize that degradation reactions leading to the loss of desirable flavor components may also involve oxidative steps and be influenced by the food environment, i.e., oxygen level. This study reports on the role of oxygen level on the stability of several classes of potentially desirable aroma compounds during storage in a water and oil medium.

MATERIALS AND METHODS

Aroma Compounds. A selection of nine aroma compounds was studied (Table 1). These compounds were selected as representing different chemical classes of compounds. The selection of aroma compounds included a thiol, an aldehyde, a diketone, a phenol, a pyrrole, an ester, and a pyrazine. Chemicals used were purchased at the highest purity from Sigma-Aldrich Chemicals. Since one objective of this work was to study the influence of matrix type on our model compounds, the compounds chosen covered a wide range of water solubility, expressed as the log of the octanol–water partition coefficient (log P). The log P values (9) for each compound are presented in Table 1. The concentrations of these compounds was chosen to be close to those found in processed food systems and are shown in Table 1. Solutions of model compounds were prepared by adding calculated volumes of each aroma compound to the desired matrix volume while being stirred. Five milliliters of this stock solution was then pipetted

* Address correspondence to this author. Tel.: 612-624-3201. E-mail: lecle003@umn.edu.

[†] University of Minnesota.

[‡] Nestlé Product Technology Center Orbe.

Table 1. Model System Composition (MCT or Water Matrix) Being Stored and Ions Used for SIM Monitoring of Each Compound

aroma compd	abbrev used in figures	log <i>P</i> ^a	concn (ppm, v/v)	ions monitored by MS (SIM mode)	
butanedione	DIAC	-1.34	1538	86	43
acetaldehyde	ACET	-0.17	3846	44	29
2-ethylpyrazine	ETHPYR	0.98	154	107	80
2-methylbutanal	2-METB	1.23	769	57	41
ethanethiol	ETSH	1.27	231	62	47
furfuryl acetate	FURFACET	1.45	769	140	98
dimethyldisulfide	DMDS	1.87	154	94	79
4-vinylguaiaacol	VINYLGUA	2.24	2000	150	135
furfuryl pyrrole	FURFPYR	2.5	385	147	81

^a Log *P* values obtained from ref 9.

into 20 mL headspace vials for storage. Four vials of each sample for each sampling period were prepared; three of which were analyzed. The fourth sample was held in reserve for other analysis if needed.

Solutions of the Model Aroma Compounds. Two solvents were studied: water (distilled water, pH ~7, abbreviated W) and medium chain triglycerides oil (MCT, Delios V from Cognis/Grünau; fully saturated, shelf-stable oil).

Sample Packaging for Storage and Analysis. Vials containing sample to be stored in ambient air were immediately closed with septa previously baked to avoid any odor contamination and then Gerstel Autosampler caps. Vials containing sample to be stored in a low-oxygen environment were taken quickly to an anaerobic glove box for gas flushing and similar closure. The glove box chamber had been flushed twice with pure argon and a third time with a mix of argon and hydrogen (90:10, respectively). The presence of a catalyst system (alumina coated palladium chloride, Stak-Pak, Coy Laboratory Products, Grass Lake, MI) insured a low-oxygen level in the chamber by reacting any residual oxygen with hydrogen to form water, which was absorbed by Drierite. The O₂ level in the glove box was monitored by gas chromatography equipped with a thermal conductivity detector (GC-TCD) during sample preparation and did not exceed 0.5%.

Previous research has shown that while the sample vial closures (septa) are very impermeable to organic volatiles, they are quite permeable to oxygen. Therefore, a second oxygen barrier was used by packaging the sealed sample vials in metallized polyester foil pouches (three side seal Malipak, 16.5 cm × 20.32 cm OD, Karpak, Minneapolis, MN). The vial-loaded foil pouches were vacuum treated and then argon flushed before final sealing. The sample environment ultimately contained <0.5% oxygen, the remainder being small amounts of nitrogen and primarily argon.

Storage of Vials. Samples in air (no pouches) and low-oxygen environments (aluminum-sealed pouches) were stored standing in an incubator at 30 °C. Reference samples to include in analysis to monitor the stability of the analytical system were frozen immediately. Sampling times were 0, 1, 2, 4, 8, and 12 weeks of storage. At sampling time, samples were transferred to a -46 °C freezer until analysis. Vials were carefully frozen in standing positions in order to avoid contamination of the septum with the liquid inside.

Method for Gas Analysis. Gas analysis of the anaerobic chamber, pouches, and vials was performed using a Hewlett-Packard gas chromatograph (HP-5890) equipped with thermal conductivity detector (TCD) and an HP-Molesieve column 30 m × 0.53 mm × 50 μm (J&W Scientific, Folsom, CA). The operating parameters were as follows: injection port, 150 °C; isothermal oven, 40 °C; detector, 175 °C; column head pressure, 5 psi; and column flow, 5 mL min⁻¹. Ten microliter samples were taken with a gastight syringe (Hamilton, Switzerland) from either pouches or vial headspace for analysis.

Analytical Method for Volatile Analysis. Extraction Method. Automated solid-phase microextraction (SPME) was used to isolate volatile compounds from the samples (Gerstell Combipal MPS 2). A 75 μm PDMS/CBX/DVB fiber was used (Supelco, Bellefonte, PA). The extraction parameters were as follows: 60 min equilibration of the sample at 55 °C, 10 min SPME sampling at 55 °C, and 5 min

Table 2. Average Level of Oxygen Determined in Pouches and Vials^a

sample	% Ar	% N	max calcd
			O ₂ %
pouch	98.3 ± 0.7	1.3 ± 0.4	0.41
vial	98.2 ± 0.8	1.5 ± 0.4	0.46

^a Values presented are averages of 20 pouches and 40 vials (average ± standard deviation.)

desorption in a gas chromatograph (GC) inlet at 225 °C. The same procedure was used through the whole experiment.

Separation and Identification. An Agilent gas chromatograph (HP-6890) equipped with a 30 m × 0.25 mm × 0.5 μm DB-Wax column (J&W Scientific, Folsom, CA) was used in analysis. The operating parameters were as follows: constant column flow control at 1 mL min⁻¹; helium as carrier gas, column head pressure 6.86 psi; split-less mode 5 mL min⁻¹ for 3 min; oven program 42 °C/5 min/6 °C min⁻¹/135 °C/20 °C min⁻¹/190 °C/8 min. A mass spectrometer (Hewlett-Packard-5972 mass selective detector) was used coupled with Hewlett-Packard ChemStation software. The parameters were set with 0.5 min solvent delay and 1.84 scan/s.

Quantification. Quantification of compounds during storage was done by MS in SIM mode. Two abundant but yet unique ions for each compound were chosen. Their respective peak areas at the GC elution time corresponding to that of the pure reference compound were summed. Ions monitored and used for integration are presented in **Table 1**. The quantitative data reported are peak areas relative to those of the corresponding compound at week 0. Samples were analyzed randomly in blocks corresponding to weeks of storage and compared to reference samples (stored frozen until analysis - week 0) analyzed within the block. Triplicate samples were analyzed.

Data Analysis. Data presented represent the average peak areas obtained at each sampling period. Data are generally expressed in terms of percentage remaining based on the peak area at time 0. In a second data treatment, a regression function was calculated. In this treatment the data were linearized using logarithmic values. Results were then presented as log(% remaining) vs time. For comparison purposes, linear regression parameters (rate of loss) are presented in bar graphs for a given matrix or a given compound.

In addition, analyses of variances (ANOVA) were conducted with the R.2.0.1 package on the rate of losses obtained for each system. We studied the influence of the type of matrix, atmosphere, and compound as sources of variability.

RESULTS AND DISCUSSION

Efficiency of Gas Flushing. All pouches were tested for oxygen content, and at least two vials in each pouch were also tested. If one vial had a high-oxygen level (>0.5%), a third vial was analyzed. Due to the low level of oxygen (<0.5%) in the samples and some tailing of the argon peak, the oxygen peak could not be directly measured. The oxygen content of a sample was, therefore, calculated based on the quantity of nitrogen detected: assuming the amount of oxygen was approximately equivalent to a quarter of the amount of nitrogen. Consequently, these calculated values are the maximum possible amounts since the oxygen in the anaerobic hood should have been consumed by the catalyst system and thus, not be 25% of the nitrogen level but less. A sampling of the results is shown in **Table 2**.

As presented in **Table 2**, maximum calculated oxygen levels (a quarter of the highest nitrogen value) were consistently below 0.5% oxygen. The results were consistent between pouches and vials of a given week and throughout storage.

Initial Headspace Levels of Model Volatiles. Since all of the loss data to be reported and discussed later have been normalized to percentage remaining over time, it is useful to

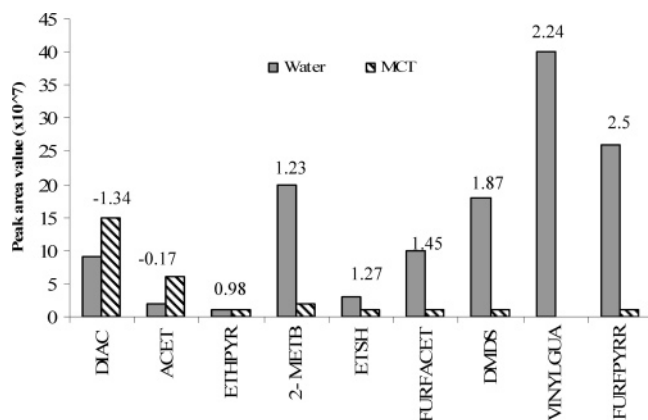


Figure 1. Peak areas for model compounds in water and MCT (air environment) at initial time. (Log P values (9) are inserted above compound.)

present an overview of the GC profile of the model compounds at time 0. As one would expect, the peak areas varied greatly with model compound and matrix. This is partly because the model had varying amounts of individual volatiles (**Table 1**). However, peak areas also reflect the solubility and volatility of each compound in each matrix (water or oil), the extraction efficiency of the SPME fiber for each compound, and the competitive binding of aroma compounds for the SPME fiber (matrix effect on SPME recovery). It is well-known that fibers will preferentially bind certain volatiles at the expense of others (10, 11).

The effect of the system matrix on peak area is related to the log P of each volatile. Compounds such as 4-vinylguaiacol (log $P = 2.24$) will show much lower peak areas in an oil vs a water system (**Figure 1**). Compounds that have higher Log P values have less solubility in water and thus are forced into the headspace thereby giving higher headspace responses in the water matrix, and the converse occurs in the oil system. Compounds with Log P values close to 0 would be expected to show similar peak areas across matrixes (assuming they have equal affinities for the SPME fiber and volatility) (**Figure 1**). Compounds with such Log P values have approximately equal solubility in water and oil systems. However, this is not observed for acetaldehyde (log $P = -0.17$). This reflects that, as noted earlier, the peak area response is not influenced solely by the solubility of each compound but also influenced by the SPME fiber affinity for each volatile as well as the total volatile load and composition on the fiber, as documented by Nongonierna et al. (10) and Roberts et al. (11). The use of a single chemical property cannot predict the initial peak area values obtained by static headspace SPME extraction.

Stability of Model Compounds during Storage. The effects of two parameters (type of matrix and presence or absence of oxygen) were studied in this work. The effects of each of these parameters on volatile stability are presented and discussed below.

Water vs Oil Systems (Modeled by MCT). The first comparison of volatile stability is in an oil system (MCT) vs a water system. Since it is impossible due to space limitations to present plots of percent loss vs time for all of the compounds included in this study under all storage conditions, only selected data are plotted to illustrate a range in behaviors. Loss rates for all compounds across all storage conditions are presented in later figures. In **Figure 2** one can see that furfuryl acetate is much more stable during storage in MCT than in water. Furfuryl acetate concentration in the water matrix dropped below the

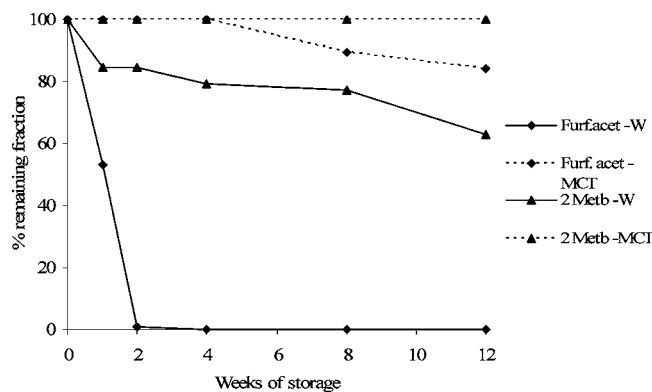


Figure 2. Relative amount of furfuryl acetate and 2-methylbutanal upon storage in water (W) and medium chain triglycerides (MCT) in air atmosphere.

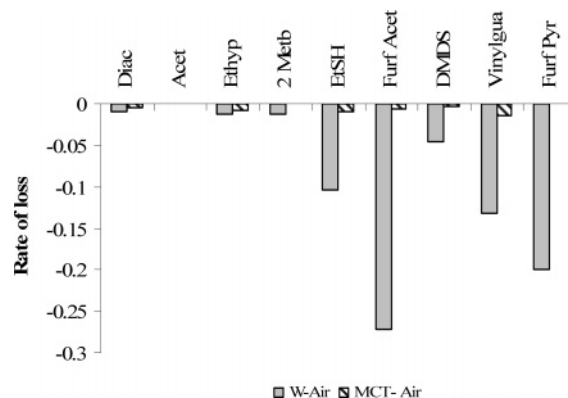


Figure 3. Rates of loss after 12 weeks of storage of individual model volatiles stored in water (W) and medium chain triglycerides (MCT) in ambient oxygen level environment. (y-axis units are rates of loss in log(%)/week, calculated from linearization of log(% remaining fraction)).

detection limits in only two weeks while ca. 82% remained after 12 weeks storage in MCT (**Figure 2**). While 2-methylbutanal is also more stable in MCT than water, the difference between matrixes is much less pronounced.

The rate of losses for each compound obtained from the linearized (log-transformed) percentage data for samples stored in MCT and water matrixes (and air) is presented in **Figure 3**. Overall, the rate of volatile loss was less in MCT than in water (stored in air), the extent of volatile loss depending on the individual compounds (although losses in the water system were in some cases low). For the water matrix, stability decreased in the order of acetaldehyde < 2,3-butanedione \approx 2-ethylpyrazine \approx 2-methylbutanal < ethanethiol \approx dimethyldisulfide < 4-vinylguaiacol < furfuryl pyrrole < furfuryl acetate. Those results are in good agreement with the results obtained by Chen et al. (7) when studying flavor stability in methylcellulose and fat systems. In their study, sulfur compounds degraded to a large extent in the absence of oil and to a lesser extent when the matrix contained 5% oil. In addition, the authors also found that the effect of matrix on storage stability was significantly compound dependent.

While a detailed investigation of the degradation mechanisms for the individual compounds was not the subject of the current study, the differences in compound stability point to very different pathways and parameters that govern their stability. Water as an aroma carrier may be a reactant in aroma degradation itself, like in the hydrolysis of esters (furfuryl acetate), or act as catalyst in protonic reactions (e.g., condensa-

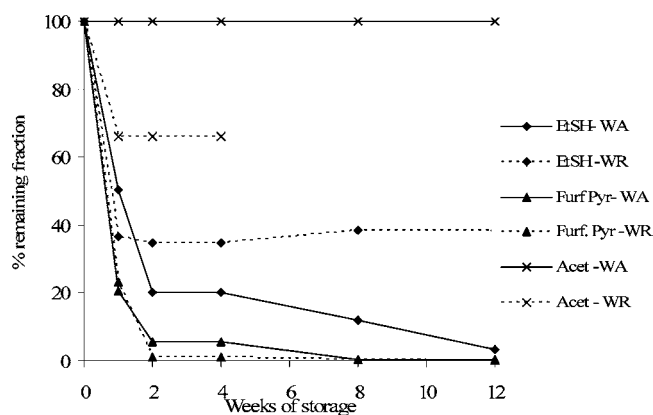


Figure 4. Influence of storage atmosphere on the percent of ethanethiol (ETSH), furfuryl pyrrole (FURFPYR), and acetaldehyde (ACET) remaining in sample headspace during storage (W = water; R = argon; A = air).

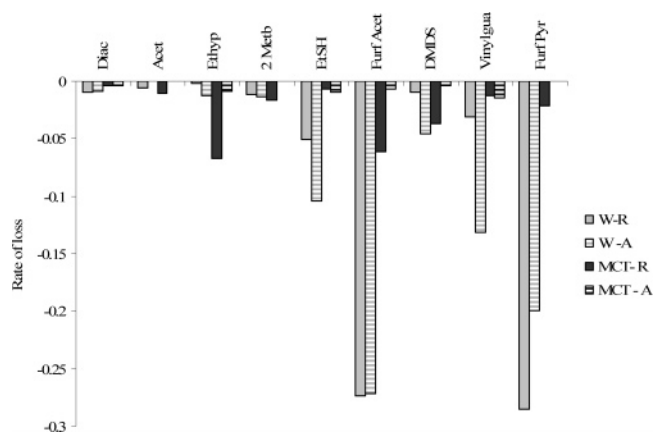


Figure 5. Rates of loss of individual model volatiles during storage (W = water; MCT = medium chain triglycerides; R = argon; A = air; y-axis units are rates of loss in log(%)/week, calculated from linearization of log(% remaining fraction)).

tion of pyrroles with aldehydes). Oil as aroma carrier, particularly a shelf-stable fully saturated triglyceride like MCT, is not directly involved in such degradation reactions (12).

As presented in **Figure 3**, the model aroma compounds were quite stable in the MCT matrix. Acetaldehyde, 2-methylbutanal, and furfuryl pyrrole did not degrade to any measurable amount over the entire storage period. The rate of loss increased in the following order: acetaldehyde < diacetyl \approx dimethyl disulfide < furfuryl acetate < ethylpyrazine \approx ethanethiol < vinyl guaiacol.

Influence of the Presence of Oxygen. The influence of oxygen on the losses of selected compounds (ethanethiol, furfuryl pyrrole, and acetaldehyde) during storage is presented in **Figure 4**. These compounds were chosen to illustrate their very different behaviors in the two atmospheres. Samples labeled "air" have been stored in an air environment while those labeled as being stored in "argon" have been flushed with argon and have less than 0.5% residual oxygen (**Table 2**).

The absence of oxygen resulted in a minor reduction in the loss of ethanethiol (60% lost in reduced oxygen environment after 2 weeks compared to 80% lost in ambient air after 2 weeks). It is clear that oxygen reduction alone is not sufficient to prevent the degradation of sulfur compounds (in our model systems). Furfuryl pyrrole was lost very quickly in the water matrix (>90% within 2 weeks), and the change of atmosphere showed no observable difference on the rate of loss. A similar pattern was observed for furfuryl acetate. To the contrary,

Table 3. Results of ANOVAs Conducted for Each of the Individual Compounds^a

compd name	type of matrix	type of atmosphere
acetaldehyde	*	*
ethanethiol		*
2-methylbutanal	*	
diacetyl		*
dimethyl disulfide		
ethylpyrazine		
furfuryl acetate	**	
furfuryl pyrrole	**	
4-vinylguaiacol		*

^a Double asterisks (**) represent a significant effect of the source of variation at a 1% level, and a single asterisk (*) represents that at a 5% level. A blank entry represents no significant influence of the source of variation at these levels.

Table 4. Results of ANOVAs Conducted for Each Type of Oil^a

type of matrix	type of compd	type of atmosphere
water	**	*
MCT		

^a Double asterisks (**) represent a significant effect of the source of variation at a 1% level, and a single asterisk (*) represents that at a 5% level. A blank entry represents no significant influence of the source of variation at these levels.

acetaldehyde was lost much more quickly from the sample headspace when stored in a low-oxygen environment (30% lost after 1 week in low-oxygen environment vs no loss in ambient air environment). While this may not seem rational, there may be reasons for this result since it is likely that different reactions take place in the presence or absence of oxygen. Storage under a low-oxygen environment may produce degradation products from other volatiles that react with the acetaldehyde resulting in its loss. These three aroma compounds illustrate the diversity of influences of the presence of oxygen on the storage stability of flavor compounds.

A more global view of the influence of oxygen level on volatile stability during storage is presented in **Figure 5**. In this figure, the rates of loss are presented for all compounds in both the water and MCT matrixes. This data overview supports the conclusion that volatiles are typically less stable in an ambient oxygen level environment than a low-oxygen environment, regardless of the matrix system they are diluted in. If a reduction of loss is observed for a compound in water at low-oxygen environment compared to water at ambient air, it is mostly the case as well when diluted in oil, and vice versa.

An additional observation is that some volatiles are equally stable in both environments: their degradation is unaffected by the presence or absence of oxygen as is the case for furfuryl acetate which is likely lost via hydrolytic reactions in the water system. However, the anticipated corresponding end product of this hydrolysis, furfuryl alcohol, was not detected in the stored systems. No degradation mechanisms could be proposed for the water or the oil system based on our data since no degradation products were detected by GC-MS in full scan mode.

Statistical Analysis. A statistical two-way ANOVA was conducted. The results showed that the simple factors have an influence on the rate of compound loss at a 5% significance level. A two-way interaction was not found to have a significant effect on the rate of degradation according to this analysis. Additional ANOVAs were conducted on the data set to focus

on individual compounds and on individual factors to detail their relative influence. Results are grouped in **Tables 3** and **4**.

Statistical analysis confirms the trends observed in the study and presented in the previous graphs. They show in addition that the effects of each of the studied parameters differ in importance for given compounds, reinforcing the idea that aroma compounds vary in stability depending upon the matrix they are dispersed in.

Modified atmosphere packaging is typically done using N₂ with or without CO₂. Product environment (headspace gas) has been used to extend shelf life from a microbiological point of view, as it slows down the growth of spoilage organisms (e.g., meat products). It is also frequently used to limit lipid oxidation in numerous food products (13) thereby reducing the formation of off-flavors, e.g., in citrus beverages, meat products, snack foods, etc. However, most of this past work has focused mainly on the appearance of defects such as color or off-flavors during storage rather than the stability of the desirable aroma components (14–16). Our study suggests that storage under a normal oxygen environment may be detrimental to the flavor of a food due to the enhanced loss of some desirable flavor notes and even reduced oxygen environment may only retard the degradation of some of these notes or may have no impact at all (e.g., esters, pyrroles). Therefore, in order to design an effective flavor protection approach for a food product, the desirable key aroma compounds that need to be preserved as well as their predominant degradation pathways need to be known. Strategies to further protect some of the aroma compounds from degradation require more insight into the mechanisms as well as research on further stabilization. The effect of antioxidants on aroma stability needs to be investigated as it has been observed that a low-oxygen atmosphere generally provided more stability. It is our opinion that the protection of desirable, characterizing aroma compounds could be as important in extending shelf life as the inhibition of off-flavors.

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Received for review August 16, 2006. Revised manuscript received November 10, 2006. Accepted November 12, 2006.

JF062362H