# Formation of α-terpineol in Citrus Juices, Model and Buffer Solutions

E. Haleva-Toledo, M. Naim, U. Zehavi and R.L. Rouseff



**ABSTRACT**

**The formation of α-terpineol from its putative precursors in citrus juice (d-limonene and linalool) was investigated in juice, buffers and model solutions. α-Terpineol content was higher in commercial lemon juice than in orange or grapefruit juices. Its content exceeded its taste threshold of 2.5 mg/L in orange juice stored for 1 month at 35 °C. During storage of homogenized model solutions fortified with d-limonene or linalool, α-terpineol was simultaneously formed and degraded, especially at 45 °C and its formation was strongly dependent on pH. Linalool was a more reactive substrate than limonene for α-terpineol formation; the protonation in linalool was faster than in limonene. However, since there was more limonene than linalool in citrus juices, α-terpineol appeared to have been formed to about the same extent from both precursors.**

**Key Words: α-terpineol, linalool, limonene, citrus, model solutions**

## INTRODUCTION

α-TERPINEOL, A DELICATELY FLORAL, LIlac-like flavor (Clark and Chamblee, 1992) occurs in many essential oils (Ravid et al., 1995). It is a desirable flavor in many plants and fruits, such as apricot and mango (Chassagene and Crouzet, 1995), whereas in others it is perceived as an off-flavor described as terpentine-like, camphoraceous, stale, musty and pungent (Lee and Nagy, 1996). α-Terpineol has been proposed as an indicator for predicting storage time of orange juice (Askar et al., 1973b). It is a degradation product of essential oil components in some fruit juices. During the processing of canned cherries, α-terpineol content increases while d-limonene decreases (Pierce et al., 1996). It occurs in aged orange juice (Kirchner and Miller, 1957; Rymal et al., 1968) and in canned orange juice (Tatum et al., 1975). α-Terpineol, along with 4-vinyl guaiacol (PVG) and 2,5-dimethyl -3(2H)-furanone (DMHF, furaneol), have been proposed as major objectionable flavors in canned orange juice. After 13 days at 32 °C in a soft package, orange juice was described as stale and musty due to the linear formation of α-terpineol with storage time (Durr et al., 1981). When added to commercial orange juice, α-terpineol imparted a stale, musty or piney odor with a detection threshold of 2.5 mg/L.

The precursors of, and mechanisms by which PVG and DMHF are produced in orange juice have been proposed (Peleg et al., 1992; Haleva-Toledo et al., 1997), but less has been published on α-terpineol. Moshonas and Shaw (1989) noted that during 8 months of storing aseptically packaged orange juice, a gradual decrease occurred in several flavor components, whereas α-terpineol and furfural increased. Durr et al. (1981) identified decreased contents of limonene, linalool, neral and some other essential oil components during storage of orange juice, while the content of α-terpineol increased. The increase in stored citrus juice appeared to be more dependent on the storage temperature than on the initial d-limonene content. In model solutions of orange juice, α-terpineol was formed from d-limonene or linalool (Askar et al., 1973a), though quantitative data were not provided. The effects of pH, which varies among citrus juices, and temperature on the formation of α-terpineol from its precursors have never been reported.

We studied citrus juices, a model solution, and different buffer solutions with the objective of quantifying the formation of α-terpineol from d-limonene and linalool, its most likely precursors in stored citrus products.

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## MATERIALS & METHOD

### Preparation of citrus juices, model and buffer solutions

Commercial single-strength orange (SSOJ), grapefruit and lemon juices, hot-filled in 250-mL glass bottles, were purchased from "Rimon" (Kibbutz Givaat-Brenner, Israel). Bottles of the juices were stored for 2 wk at 45 °C. In addition, SSOJ was preserved with 500 mg/L sodium benzoate and stored in closed 50-mL brown bottles for 6 and 8 wk at 35 and 45 °C. Half of the stored bottles were fortified with 8 mM cysteine.

Model orange juice solutions (MOJ) containing the major sugars and amino acids were prepared according to Peleg et al. (1992). To increase the solubility of d-limonene and linalool, they were added to the solutions following the procedure of Ben-Aziz et al. (1971): 2 g of Tween 80 with 2 g of d-limonene or 0.002 g of linalool were dissolved in 50 mL chloroform; this solution was placed under vacuum at 35 °C until the chloroform evaporated. The remaining solution was added to the MOJ to a final concentration of 1g/L d-limonene or 10 mg/L linalool. Each solution was homogenized for 20 min at 500 bars (APV Rannie AS homogenizer, Copenhagen, Denmark). The solutions were stored in sealed 20 mL brown bottles at 25, 35 and 45 °C for 2, 4 or 6 wk. In another experiment, 2.5 and 8 mM L-cysteine or 10 mM N-acetyl-L-cysteine were added to homogenized MOJ that contained 2 g/L d-limonene or 22.8 mg/L linalool.

Buffer solutions were either citrate phosphate (0.1M, pH 2.8, 3.8 and 6) or Tris (0.1M, pH 8.4). d-Limonene and linalool were added to the solutions as described (10 mL of d-limonene or linalool and 10 mL of Tween 80 dissolved in 10 mL of chloroform) to provide a final concentration of 1 g/L d-limonene or 50 mg/L linalool. The solutions were then stored in 50-mL brown bottles at 4 or 35 °C for 4 wk.

### Extraction and chemical analyses

α-Terpineol was extracted from buffer solutions according to Moshonas and Shaw (1987). Buffer or model solutions (50 mL) were distilled (by heating to 60 °C under reduced pressure of 60-mm Hg for 20 min, to complete distillation, then condensing with ice water and a liquid-nitrogen trap). Distillate samples (2 to 5 µL) were injected in a GC (Hewlett-Packard 5890) equipped with a flame-ionization detector and a 60-m fused silica nonpolar capillary column loaded with cross-linked 5% phenylmethyl silicone (film thickness 1.0 µm, 0.32 mm i.d., Rtx-5, Restex Corp. Bellefonte, PA). Nitrogen was used as carrier gas. Conditions were: split ratio 1:20, flow rate 1.5 mL/min, injector and detector at 275 °C. The oven program was set

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at: 50 °C (1 min), at 17 °C/min to 150 °C (1 min) at 7 °C/min to 200 °C (1 min), and at 10 °C/min to 220 °C (3 min). A 100 mg/L solution of butanol was used as internal standard for analyses of α-terpineol and linalool.

For citrus-juice extraction analysis of α-terpineol in commercial orange, grapefruit and lemon juices, samples were centrifuged (15000 × *g*, 4 °C, 15 min). Supernatant or MOJ (5 mL) was applied to a C18 Sep-Pak cartridge (Water Assoc. Milford, MA) which had been preconditioned with 2 mL methanol and 5 mL water. Cartridges were then washed with 2 mL water and eluted with 1.5 mL methylene chloride. Sodium sulfate was added to remove the traces of water, and 2 µL were then injected to GC (Hewlett-Packard 6890) equipped with the capillary column, using nitrogen as carrier gas. Conditions were: split ratio of 1:10, flow rate of 1 mL/min, injector and detector temperatures of 275 °C. The oven program was set at: 50 °C (5 min), at 10 °C/min to 150 °C (1 min), at 4 °C/min to 200 °C (2 min), and at 10 °C/min to 220 °C (5 min). Butanol was used as an internal standard.

**Data analysis**: Results were analyzed by one or two-way analysis of variance (ANOVA) using JMP statistic computer program (SAS Institute Inc.). Significance level was accepted at least at the $p < 0.05$ level.

## RESULTS & DISCUSSION

### α-Terpineol in commercial citrus juices

The presence of α-terpineol in commercial lemon, grapefruit and orange juices was found before and after accelerated storage at 45 °C for 2 wk (Fig. 1a). In lemon juice, however, the level of α-terpineol before storage was about 10-fold higher than its 2.5 mg/L taste threshold, whereas in orange and grapefruit juices, its content was below taste threshold. In addition, the relative content of α-terpineol in lemon oil was higher than in orange and grapefruit oils (Fig. 1b). α-Terpineol may therefore be a desirable natural component of lemon juice. In contrast, and confirming previous reports (Moshonas and Shaw, 1989), α-terpineol is undesirable in orange and grapefruit juice aroma and it increases during storage. In orange juice, its content reached the taste threshold after 2 wk at 45 °C (Fig. 1a) and was two- to threefold higher than the taste threshold after 6 or 8 wk at 35 °C (Fig. 2). Moreover, its increase was both temperature- and time-dependent (Fig. 2). In contrast, its content decreased in lemon juice during storage (Fig. 1a), probably due to conversion to other components such as cis- and trans-1,8-p-menthanediol (Tatum et al., 1975).

### Formation of α-terpineol in model and buffer solutions

Cold-pressed orange oil contains about 96% terpene hydrocarbons (mostly d-limonene) (Shaw, 1979) and the amount of oil in citrus juice varies with juicing and other processing and packaging conditions. Moshonas and Shaw (1989) found 170 mg/L oil in aseptically packaged orange juice before storage. d-Limonene has a very limited solubility of 14 ppm in water (Clark and Chamblee, 1992). Citrus juices contain neutral emulsifiers and are somewhat homogenized during processing. In preliminary experiments, the addition of d-limonene to MOJ without emulsifiers and without homogenization resulted in two separate phases, not seen in the homogenized system. Consequently, we used homogenized MOJ in both, d-limonene- and linalool-containing solutions.

Storage of homogenized MOJ fortified with 10 mg/L linalool for 6 wk at 25, 35 and 45 °C resulted in linalool degradation, whereas α-terpineol formation occurred only at 35 and 45 °C (Fig. 3a). After 2 wk at 45 °C and 6 wk at 35 °C, the degradation of α-terpineol was faster than its formation from linalool.

Storage of homogenized MOJ fortified with 1g/L d-limonene for 6 wk at 25, 35, and 45 °C resulted in α-terpineol accumulation, which increased during the first 4 wk and then either continued to increase slightly 25 °C or decreased (35 and 45 °C) (Fig. 3b). Under acidic conditions, monoterpenes such as d-limonene, linalool and α-terpineol degrade to other terpenes via the hydration of double bonds, dehydration, cyclization and hydrolysis of esters (Clark and Chamblee, 1992). d-Limonene is converted mostly to α-terpineol, whereas linalool is converted to α-terpineol plus citral (mixed isomers of neral and geranial). Apparently, both (+)-α-terpineol and (-)-enantiomers occur in citrus (Ravid et al., 1995). However, the coniferous-like odor of the (-) enantiomer appears to be more dominant in stored citrus juice (Tatum et al., 1975) than the floral character of the (+) enantiomer. Under acidic and prolong storage citrus products, the α-terpineol may be converted to other compounds, such as 1,8-p-menthandiol (Tatum et al., 1975). This reaction is probably faster at higher temperatures, as well as under acidic conditions (such as in lemon juice).

In our results, α-terpineol was converted to unidentified products faster than it was formed, especially at 45 °C, from both linalool and limonene.

Our conditions—homogenization and use of Tween 80, probably resulted in a more thoroughly homogenized solution than commercial orange juice. The acid catalysis in

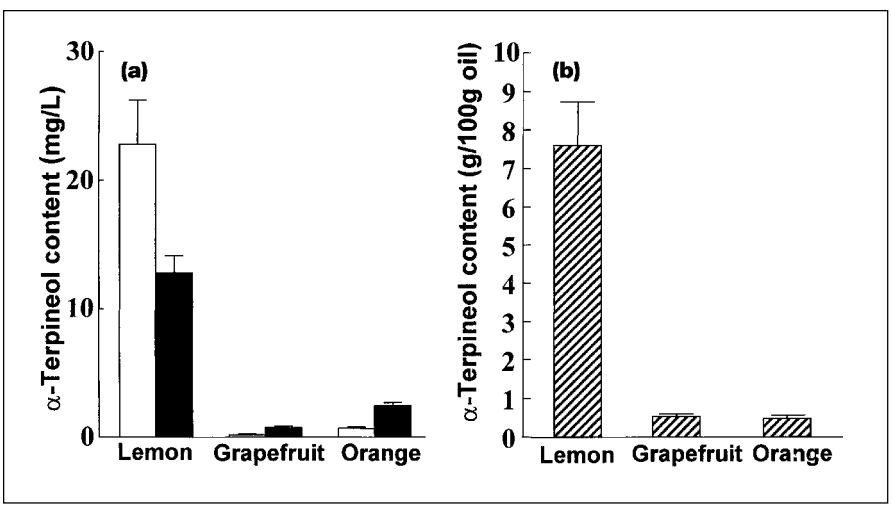


**Fig. 1—(a) α-Terpineol content in lemon, grapefruit and orange juice before (open bars) and after (solid bars) storage for 2 wk at 45 °C and (b) the content of α-terpineol in oil (hatched bars). The amounts of oil were 300 mg/L in lemon juice, 40 mg/L in grapefruit juice and 140 mg/L in orange juice. Means and SEM of 3 samples, each analyzed twice by GC.**

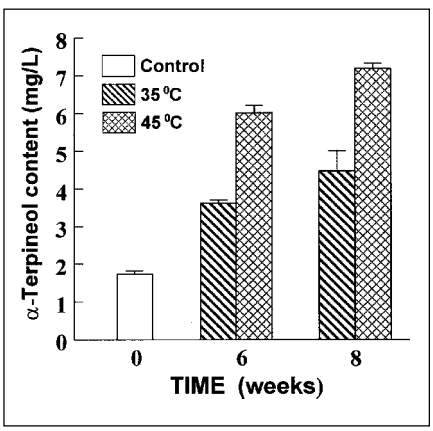


**Fig. 2—Formation of α-terpineol in commercial orange juice stored for 6 and 8 wk at 35 and 45 °C. Means and SEM of 3 samples, each analyzed twice by GC. Control samples were kept at 4 °C.**

our MOJ may have been more effective in the degradation of d-limonene and linalool than that occurring in citrus juice, leading to accelerated degradation of α-terpineol in our MOJ. In a separate experiment (data not shown), using a similar incubation without homogenization and without Tween 80, these degradation processes were much less apparent.

In our MOJ experiments and confirming other studies (Askar et al., 1973a), the formation of α-terpineol from linalool was faster than its formation from d-limonene at 35 °C. However, the content of d-limonene in orange juice is about 10- to 100- fold higher than that of linalool (Moshonas and Shaw, 1989). In our results, linalool in commercial orange, grapefruit and lemon juices was 1.32, 2.97, and 1.42 mg/L, respectively. The respective d-limonene contents were 46, 12.8, and 125 mg/L. Therefore, α-terpineol was apparently formed in citrus juice from both d-limonene and linalool in about the same amounts. The conversion of limonene vs. linalool to α-terpineol is complex (Fig. 3); α-terpineol is formed and degraded at the same time (Tatum et al., 1975). Kinetics and determination of the order of such reactions under these conditions, are particularly complicated and apparently unreliable (Labuza and Kamman, 1983).

Moshonas and Shaw (1989) found a higher content (11 mg/L of linalool) in aseptically packaged orange juice, which seemed to be stable during storage. In our MOJ experiments, linalool was degraded during storage. In citrus fruits before storage, it appears that during early stages of fruit development, linalool and limonene contents increased while no changes were found in α-terpineol (Kekelidze et al., 1989). According to Attaway et al., (1967), terpenes in citrus are formed from the parent compound mevalonic acid, which forms linalyl pyrophosphate, which then forms linalool—the parent terpene for all the others. However, it was shown later that α-terpineol was not a biosynthetic product in cell-free extracts of citrus flavedo converting neryl pyrophosphate and geranyl pyrophosphate to limonene (George-Nascimento and Cori, 1971; Chayet et al., 1977).

In stored citrus juices, linalool is probably formed and degraded simultaneously. Evidently, monoterpenes in citrus juices are mostly in the oil fraction. The first step of α-terpineol formation (Fig. 4) from both d-limonene and linalool is protonation. Protonation of the OH group in linalool is faster than that to the double bond in d-limonene. True, pH had a strong effect on α-terpineol formation from either d-limonene or linalool in buffer solutions stored at 4 and 35 °C for 4 wk (Fig. 5). In both cases, the formation of α-terpineol was highest at pH 2.8. At pH 3.8, the rate of formation of α-terpineol from d-limonene was five times slower (Fig. 5a) and its formation from linalool was reduced by half (Fig. 5b). This difference related to pH is relevant to citrus-juice products, since the pH was 2.8 in the commercial lemon juice, 3.5 in orange juice and 3.2 in grapefruit juice.

The effect of pH probably explains the differences in α-terpineol content in orange, grapefruit and lemon juices before storage (Fig. 1a). Although the higher acidity of lemon juice accelerates formation of α-terpineol during storage, concomitant degradation to other products such as trans- and cis-1,8 tepinene ,1,4- or 1,8-cineole is likely to result in decreased α-terpineol content.

As mentioned, α-terpineol, along with

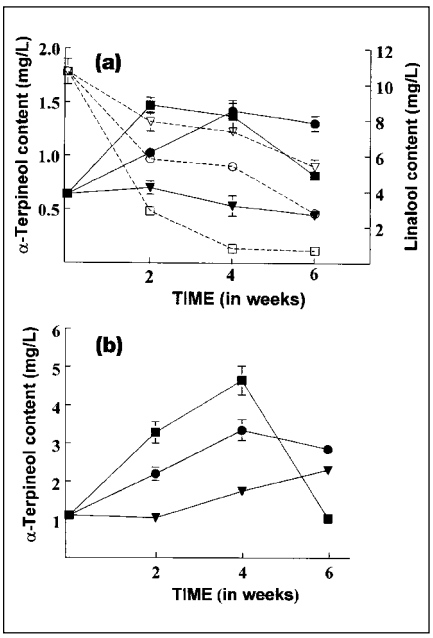


**Fig. 3—Formation and degradation of α-terpineol in MOJ solutions stored for 6 wk at 25 °C (△,▲), 35 °C (○,●) 45 °C (□,■). (a) Formation and degradation of α-terpineol (filled symbols), and the degradation of linalool (open symbols) in MOJ solutions fortified with 10 mg/L Linalool. (b) Formation and degradation of α-terpineol in MOJ solutions fortified with 1g/L d-limonene. Means (SEM of 3 samples analyzed by GC). Where SEM values not shown, they were too small to be seen.**

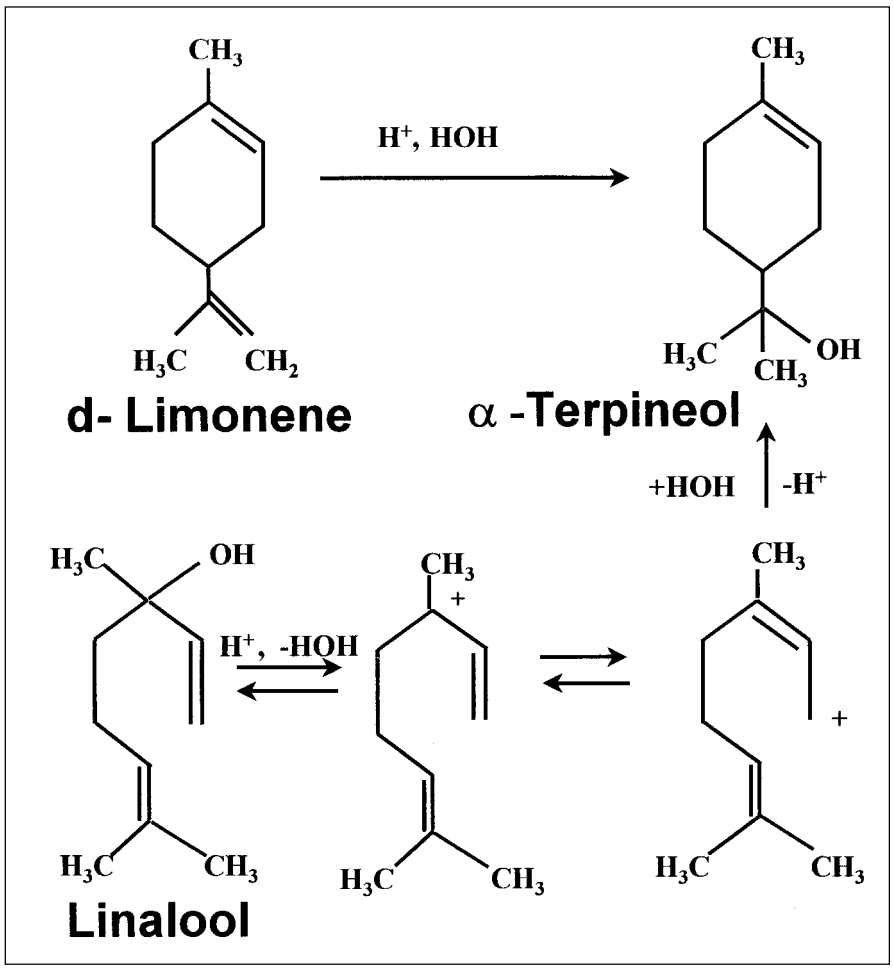


**Fig. 4—Pathways for α-terpineol formation from linalool and d-limonene.**

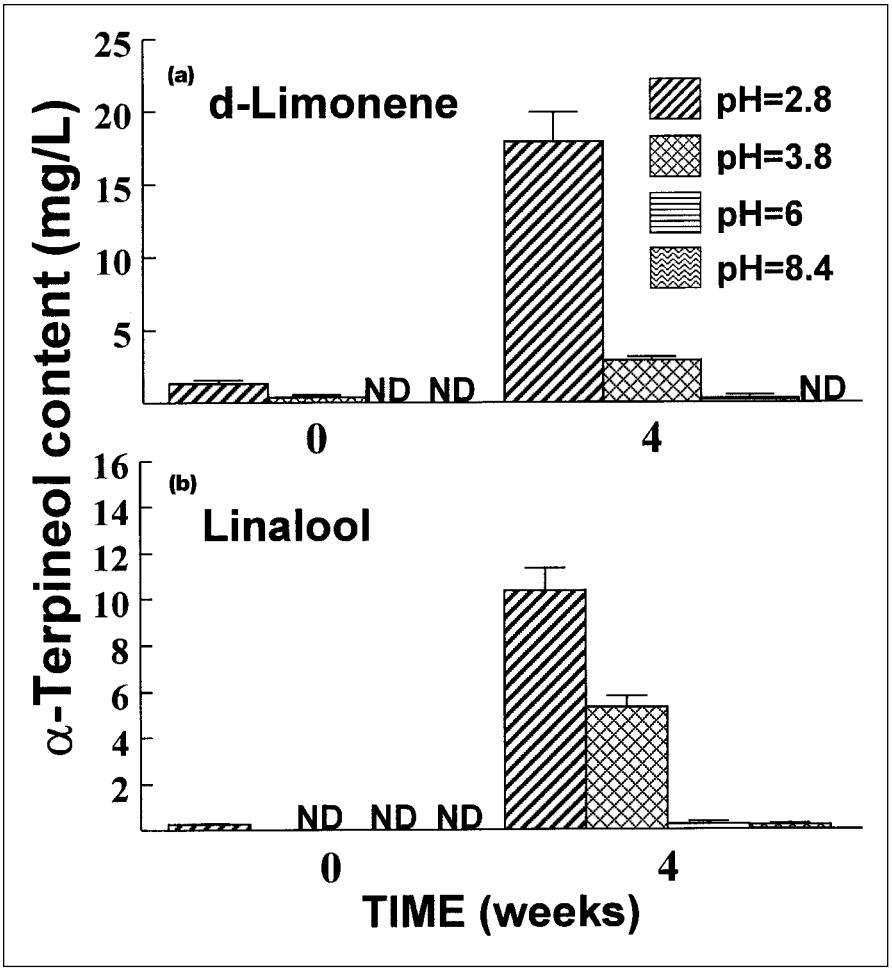


**Fig. 5—Effect of pH on α-terpineol formation from d-limonene (a) and from linalool (b) in homogenized buffer solutions stored 4 wk at 35 °C. ND = not detected. Means and SEM of 3 samples, each analyzed twice by GC.**

PVG and furaneol are objectionable flavors in orange juice. Thiol fortification has reduced furaneol and PVG content in pasteurized and stored orange juice, and improved juice acceptance (Naim et al., 1994; Naim et al., 1997). As we expected fortification with cysteine and acetyl cysteine had no effect on α-terpineol content in stored orange juice or in stored MOJ fortified with either d-limonene or linalool (data not shown). Furthermore, experiments with MOJ solutions suggested that PVG and furaneol underwent notable significant degradation during storage (Peleg et al., 1992 ; Haleva-Toledo et al., 1997). Due to its stability, α-terpineol, may be a significant off-flavor in orange juice stored for extended times. Note that GC-olfactometry analysis (Naim et al., 1998) has suggested that in addition to PVG, furaneol and α-terpineol, other unidentified off-flavors were formed during storage.

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