Impact of Oxidized Off-flavor of Ice Cream Prepared from Milk Fat

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ABSTRACT: The influence of light-induced oxidation on ice cream quality was investigated by monitoring hydroperoxide formation of the oil fraction, sensory evaluation for oxidized off-flavor, and gas GC–MS analysis of developed volatile aldehydes following steam distillation/extraction of ice cream. Two ice cream samples containing 14.3% milk fat prepared from different milk fat materials, and a sample with 1000 ppm tocopherol added were exposed to 650 lx fluorescent light at −20°C. Ice cream prepared from each milk material showed a different time dependence for peroxide formation, and the addition of tocopherol reduced hydroperoxide formation. The correlation coefficient (r^2) between the PV and the sensory score was 0.483 ($n = 9$), and the r^2 between the total concentration of 10 volatile aldehydes developed during light exposure and the sensory score was 0.365. Among the 10 aldehydes, however, the highest correlation observed was between the amount of *trans*-2-decenal and the sensory score ($r^2 = 0.864$, $n = 9$), and lowest correlation was between the amount of hexanal and the sensory score ($r^2 = 0.302$, $n = 9$). These results suggest that exposure of ice cream in the frozen state to fluorescent light produces a unique composition of volatile aldehydes related to flavor deterioration induced by photooxidation of the milk fat in ice cream.

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KEY WORDS: Hexanal, ice cream, photooxidative stability, sensory evaluation, *trans*-2-decenal, volatile aldehyde.

Ice cream is a frozen dairy product that is consumed worldwide. It contains approximately 8–15% fat, which contributes importantly to the desired characteristics of ice cream: a smooth, creamy, and soft mouth-feel and good flavor (1). Ice cream has some unique characteristics, such as a solid fat network, that have been studied by many researchers. Schmidt and Smith (2) reported the effects of processing conditions on the physical properties of ice cream. Goff and others (3) studied the influence of milk components on the physical properties of ice cream. Most of the research on ice cream has focused on its physical characteristics and sensory aspects.

Recently, most grocery stores have adopted stronger lighting on food products to show their products attractively. Since grocery store business hours are often extended, the potential risk of light-induced degradation of food products increases. Deterioration as a result of oxidation during storage has not been considered as a critical problem for ice cream, because it is usually kept in a frozen state (−18 to −20°C) (4). Ice cream contains, however, a high amount of fat compared with other food products; therefore, it might deteriorate during storage under strong lighting in grocery stores.

Light-induced off-flavors in milk have been documented in several reviews (5). Photooxidation of milk fat was assumed to be a major factor in light-induced flavor changes in milk and to be dependent on the light exposure time, storage temperature, and/or spectral wavelength (6,7). Oxidized milk fat generates hydroperoxides, which decompose to volatile compounds, such as aldehydes, ketones, and alcohols, that have been related to oxidized off-flavors (8). However, the theories regarding light-induced oxidation of fluid milk are considered inapplicable to the photooxidation of ice cream because ice cream has a higher fat content, different manufacturing process, and different storage conditions (1). Few studies have been reported of the development of light-induced oxidized off-flavor of dairy products under frozen conditions, and little is known about the mechanism of light-induced oxidation of milk fat in ice cream. To protect dairy products from light-induced degradation, containers consisting of various materials have been proposed (9). However, these container materials have some problems including higher cost and issues of biodegradability when discarded. Therefore, improving the oxidative stability of dairy products themselves is considered necessary. Antioxidants, such as tocopherols, which are derived from natural sources such as corn, soybean and palm oils, are widely used to inhibit lipid oxidation because tocopherols are not reported to have the undesirable physiological implications reported with the synthetic antioxidants BHT, BHA, and TBHQ (10). Tocopherols were reported to prevent lipid oxidation by trapping radicals generated during lipid autoxidation.

In this work, studies were conducted to investigate whether any correlation existed among the sensory evaluation of the off-flavors, quantities of volatile compounds developed, and PV produced in the milk fat of light-exposed ice cream. In addition, the effect of added tocopherol on light oxidation in ice cream was evaluated.

MATERIALS AND METHODS

Materials. Milk fat ingredients were obtained from different commercial sources in Australia (milk fat material A) and

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Japan (milk fat material B). These materials were prepared by conventional centrifugation from milk. Fat contents of milk fat material A and B were 40.0 and 84.0%, respectively, and the relative compositions of the FA were essentially the same (data not shown). Nonfat components except water of materials A and B were 4.8 and 1.8%, respectively. Commercial skim milk powder (Snow Brand Milk Products Co., Ltd., Tokyo, Japan) was used. The following were purchased and used as ingredients for the ice cream mix: glycerol monostearate (Riken Vitamin Co., Ltd., Tokyo, Japan) as an emulsifier; mixture of locust bean gum, guar gum, and gelatin (Taiyo Kagaku Co., Ltd., Yokkaichi, Japan) as stabilizers; β-carotene (Roche Holding Ltd., Basel, Switzerland) as a coloring agent; and sugar (Dainippon Meiji Seito Co., Ltd., Tokyo, Japan). As an antioxidant, commercial tocopherol material containing 49.8% δ-tocopherol (Riken Vitamin Co.) was used. All reagents for analysis were of analytical grade.

*Preparation of ice cream. S*kim milk, milk fat material, emulsifier, stabilizer, sugar, coloring reagent, and water were combined to produce 100 kg of mix. The fat content of all the mixes was adjusted to 14.3%, and the concentration of nonfat milk solids was adjusted to 11.5% by the addition of skim milk and water. The ice cream mixes made in this way contained 12.8% sugar, 0.3% emulsifier, 0.2% stabilizer, and 0.1% coloring reagent. The raw materials were dissolved and mixed well in water and pasteurized at 80°C for 60 s; the mixes were homogenized at a total pressure of 150 kg/cm^2 and a secondary pressure of 50 kg/cm^2 (homogenizer; Sanwa Machine Co., Inc., Numazu, Japan). The mixes were allowed to stand overnight at 5°C, then processed in an ice cream freezer (APV Crepaco, Inc., Chicago, IL) at −5°C. The overrun of all samples was adjusted to 70%. The ice cream samples (50 mL) were packed in white paper containers with transparent polyethylene terephthalate caps and kept at −20°C.

Samples. In this study, three model ice creams were prepared: sample 1: ice cream sample prepared from milk fat material A; sample 2: ice cream sample prepared from milk fat material B; sample 3: ice cream sample prepared from milk fat material A, with 1000 ppm tocopherol added.

Light exposure storage test. Samples of each ice cream were placed on shelves exposed to 650 lx fluorescent light from the top and kept at −20°C. The display case was illuminated by cool white fluorescent lamps mounted parallel to the shelves at a distance of 50 cm from the samples. At intervals of 0, 1, 3, 5, and 7 d, ice cream samples were removed for analysis. The quality of ice cream samples exposed >7 d was too poor for analysis. In addition, equivalent samples were held in the dark to serve as a positive control.

Extraction of oil fraction from ice cream. For each analysis, 50 mL of ice cream sample was dissolved in water saturated with salt. Hexane/ethanol (50 mL, 1:1, vol/vol) was added to this solution and then strongly vortexed for 3 min. The upper phase was collected, and the solvent was removed by rotary evaporator to obtain the oil from ice cream samples. Each sample was extracted twice.

Determination of PV on fat fraction of light-exposed ice cream. Photooxidative stability was evaluated by measuring hydroperoxides of the extracted fat of ice cream as PV. PV was determined by a colorimetric ferric thiocyanate method (11). These analyses were run in duplicate.

Sensory evaluation. Sensory evaluation of ice cream samples exposed to fluorescent light for intervals of 0, 3, and 7 d was conducted by six trained panel members. Before evaluation, a 2-h training session was held to familiarize the judges with the evaluation method. During training, the use of a reference, the sample presentation, and the manner to be used to evaluate samples were explained. In addition, the characteristics of oxidized off-flavors were discussed. The samples used during training evaluation were taken from ice cream, both fresh and exposed to light for various times, to obtain different intensities of oxidized flavor.

Trained panelists evaluated the ice cream for intensity of oxidized flavor. Ice cream samples at −12°C were served in 50 mL capacity white paper cups. The ice cream samples were served at room temperature in partitioned booths illuminated with white fluorescent lights. Filtered water (21°C) was provided to cleanse the palate of the panelists between the samples. Panelists were instructed to rest between samples to avoid fatigue. The intensity of light-induced deterioration of the samples was scored on a 10-point scale. Scoring ranged from 1, very strong oxidized flavor, to 10, no flavor defect.

Analysis of FA and volatile compounds by GC–MS. The FA composition of the oil fraction was measured by GLC as follows. An oil sample was transmethylated by potassium hydrate to obtain the FAME, which were then dissolved in *n*-hexane and injected into the gas–liquid chromatograph. The FA composition was measured under the following conditions: equipment, Hewlett-Packard HP6890 Series II (Hewlett-Packard, Palo Alto, CA); detector, FID; column, fused-silica capillary column, DB-WAX (30 m, 0.25 i.d.), 0.25 μm film thickness; (J&W Scientific, Folsom, CA); and carrier gas, helium.

Flavor isolates for GC–MS analysis were prepared through a steam distillation/extraction (12). One hundred milliliters of each ice cream sample was steam distilled at room temperature, then the distillate was extracted three times with dichloromethane. Volatile compounds were identified by GC–MS. Diphenyl was used as an internal standard. The concentration of volatile materials was measured by GLC, under the conditions described in the preceding paragraph. A QP-5000 (Shimadzu Corporation, Ltd., Kyoto, Japan) combined gas chromatograph–mass spectrometer was used to identify the compounds. The temperature of the interface was maintained at 250°C. The electron impact mass spectra were recorded at 70 eV.

Statistical analysis. Means obtained from flavor scores were subjected to ANOVA according to the Statistical Analysis System (SAS) (13). The probability for statistical significance was set at 5%.

RESULTS AND DISCUSSION

Light-induced oxidation of ice cream. The photooxidative stability of ice cream was investigated by measuring the progress

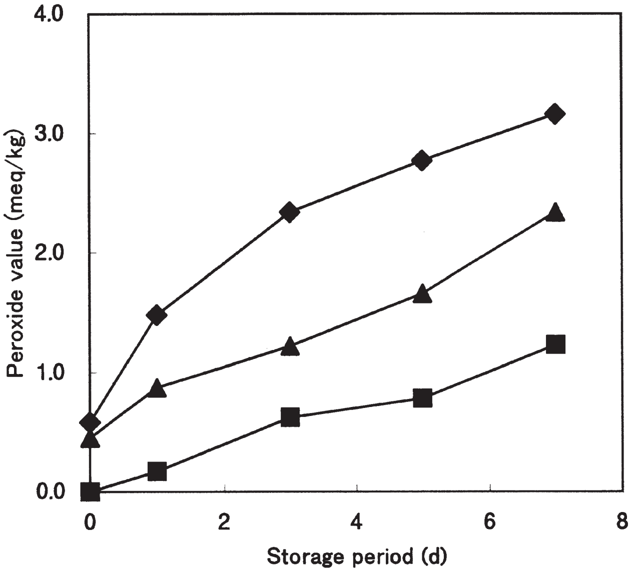

FIG. 1. Time dependence of PV of fat fraction in ice creams exposed to 650 lx fluorescent light at −20°C. (◆) Sample 1, ice cream sample prepared from milk fat material A; (■), sample 2, ice cream sample prepared from milk fat material B; (▲) sample 3, ice cream sample prepared from milk fat material A, with 1000 ppm tocopherol added.

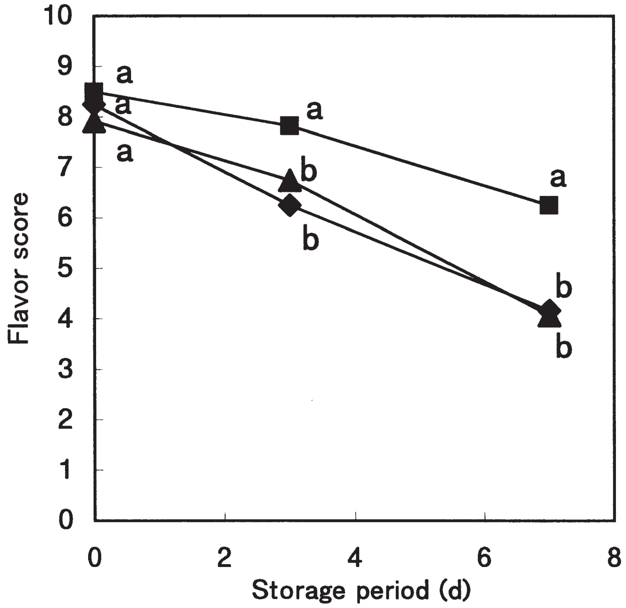

FIG. 2. Time dependence of flavor score of ice creams exposed to 650 lx fluorescent light at −20°C. Values followed by the same letters at the same storage period are not significantly different at $P < 0.05$. (◆), Sample 1, ice cream sample prepared from milk fat material A; (■), sample 2, ice cream sample prepared from milk fat material B; (▲), sample 3, ice cream sample prepared from milk fat material A, with 1000 ppm tocopherol added.

of hydroperoxide formation in ice cream samples exposed to fluorescent light for 0, 1, 3, 5, and 7 d, as shown in Figure 1. The photooxidative stability of two ice cream samples (sample 1 and sample 2) prepared from two fat materials (fat material A and B, respectively) were compared. The initial PV of the oil fractions extracted from ice cream samples 1 and 2 were 0.58 and 0.00 meq/kg, respectively. After exposure to fluorescent light for 7 d, the PV of oil fractions extracted from ice cream samples 1 and 2 were 3.16 and 1.23 meq/kg, respectively. These results indicated that the oil fraction of ice cream prepared from milk fat material A oxidized faster than that of ice cream prepared from milk fat material B. The photooxidative stability of milk is influenced by its FA and vitamin composition (14). FA and vitamin compositions are determined by feeding conditions as well as processing conditions of the component milk products (15,16). Thus, the different susceptibilities to photooxidation in ice cream samples could be due to the variation of composition in ice cream ingredients.

To prevent lipid oxidation, antioxidants are generally used. The effect of antioxidant on photooxidative stability in ice cream sample 3, which is the same as sample 1 except for the addition of 1000 ppm δ-tocopherol, was determined by monitoring PV with time (Fig. 1). After 7 d of exposure to fluorescent light, the PV of sample 3 was lower than that of sample 1, at 2.34 meq/kg. Thus, ice cream sample 1 with added tocopherol showed lower generation of hydroperoxides than the same sample without tocopherol. The PV of samples 1–3 not exposed to fluorescent light (positive control) for 7 d were the same as the initial PV (data not shown).

Sensory evaluation of light-exposed ice cream. Flavor changes during storage in ice cream samples 1, 2, and 3 exposed to fluorescent light were determined by sensory evaluation. Figure 2 shows the results of sensory evaluation of the three ice cream samples after storage for 0, 3, and 7 d. The flavor score decreased with increasing storage period, indicating that the intensity of oxidized off-flavor increased with light exposure. The scores for sample 2 exposed for 3 and 7 d were significantly higher than those of sample 1 and sample 3 for the same storage periods. No significant difference in flavor scores was observed between sample 1 and sample 3. The light-induced oxidized flavor of ice cream prepared from the same milk material showed the same flavor score regardless of the presence of tocopherol, and the flavor score of ice cream prepared from milk fat material B was higher than that from milk fat material A. The flavor defects were not detected in samples stored 7 d in the dark.

Relationship between generation of hydroperoxides and flavor scores. The relationship between the generation of hydroperoxides and flavor scores was examined, as shown in Figure 3. The flavor score of the three samples exposed to fluorescent light decreased with increasing PV. The decreasing flavor score of sample 1 was similar to that of sample 2. However, sample 3 showed a relatively lower flavor score than samples 1 and 2. A relatively low correlation coefficient ($r^2 = 0.483$, $n = 9$) was observed between PV and flavor score. The generation of hydroperoxides is the first step in the photooxidation of milk fat, and measuring PV has been used as an index for the evaluation of fat oxidation. However, determination of PV was considered inadequate for evaluating the light-induced oxidized flavor of ice cream.

Development of volatile aldehydes in light-exposed ice cream and their relation to sensory score. The development of

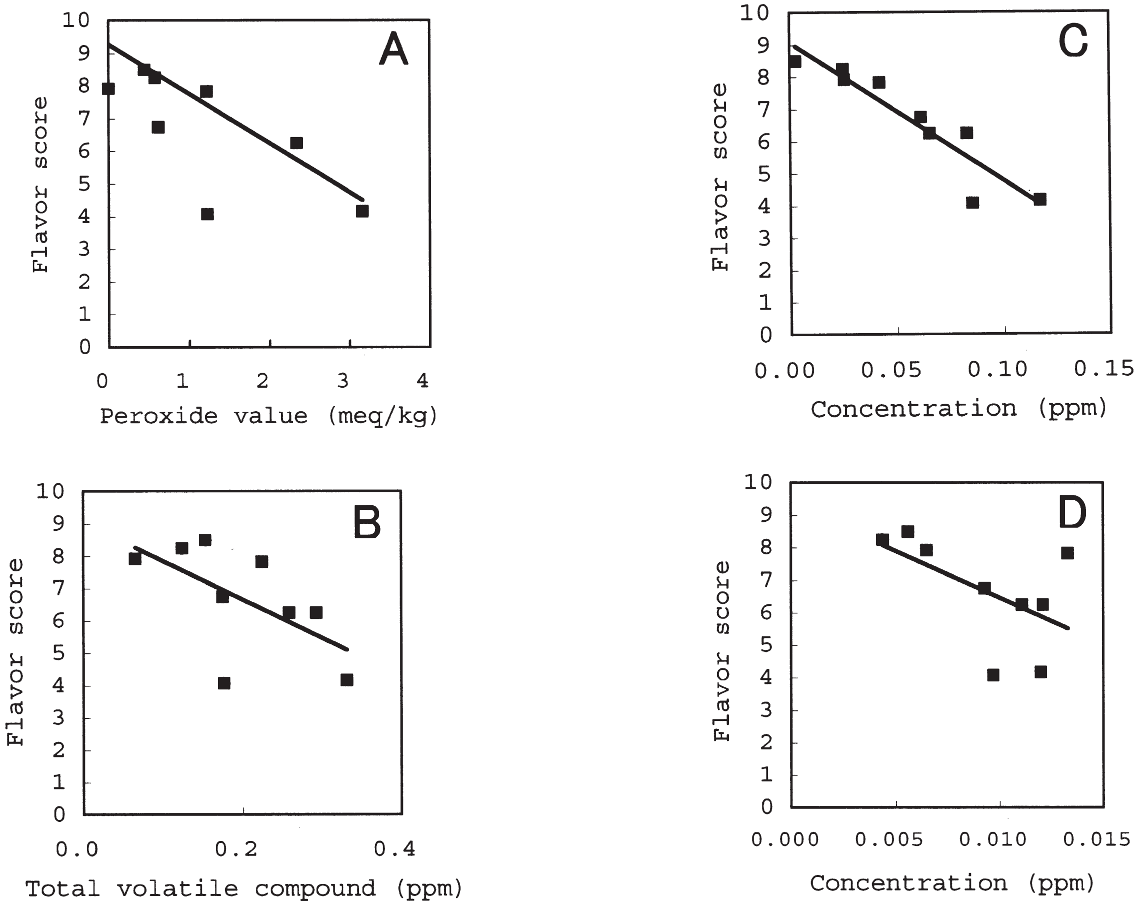

FIG. 3. Correlations between flavor score and (A) PV (r^2= 0.483, n = 9); (B) total aldehydes concentration (r^2 = 0.365, n = 9); (C) *trans*-2-decenal concentration (r^2 = 0.864, n = 9); (D) hexanal concentration (r^2 = 0.302, n = 9).

flavor compounds related to the oxidized off-flavor of ice cream samples exposed to fluorescent light was investigated. Ice cream samples 1–3 were exposed to fluorescent light for 0, 1, 3, 5, and 7 d. Among volatile carbonyl compounds, changes in the total concentration of the 10 major aldehydes—pentanal, hexanal, heptanal, octanal, nonanal, *trans*-2-heptenal, *trans*-2-octenal, *trans*-2-nonenal, *trans*-2-decenal, and 2,4-heptadienal—which have been reported to relate to the oxidized off-flavor induced from oxidation of unsaturated FA, were monitored (8). GC–MS profiles showed that the concentration of total aldehydes increased during storage (Table 1). The amount of aldehydes in light-exposed sample 1 was higher than that of sample 2, and that of sample 3 was lower than sample 1. This result showed that development of aldehydes in ice cream prepared from milk fat material A was higher than that from milk fat material B, and that of ice cream prepared from milk fat material A was reduced by the addition of tocopherol. The correlation of the sensory evaluation with the amount of total volatile aldehydes (Fig. 3B) was relatively low (r^2 = 0.365, n = 9). This result indicated that determination of total amount of aldehydes developed with photooxidaton was not enough to evaluate the flavor deterioration of ice cream.

Impact of volatile aldehydes on light-induced flavor deterioration of ice cream. To estimate the impact of volatile compounds on the flavor score, the detection of several volatile aldehydes in light-exposed ice cream samples with time was determined (Table 2). The development of hexanal in samples prepared from milk fat material A was reduced by the addition of tocopherol. However, the amount of hexanal in light-exposed sample 2 was higher than that of sample 1 when stored longer than 3 d. The amount of *trans*-2-decenal of light-exposed sample 1 was higher than that of sample 2, and that of sample 3 was lower than sample 1. This result showed that development of *trans*-2-decenal in ice cream prepared from milk fat material A was higher than in that from milk fat material B, and that development of *trans*-2-decenal in ice cream prepared from milk fat material A was reduced by the addition of tocopherol. All three light-exposed ice cream samples developed different types of volatile aldehydes. The correlation of the sensory evaluation score with 10 major volatile aldehydes was determined, as shown in Table 3. The unsaturated FA related to the generation of these aldehydes are indicated in Table 3 (17). Among volatile aldehydes, the highest correlation coefficient was observed between flavor score and *trans*-2-decenal (r^2 = 0.864), and the

TABLE 1
Development of Volatile Aldehydes in Ice Cream Exposed to Fluorescent Light

Sample number[a]	Storage period (d) 0	1	3	5	7
	Total concentration (ppm)				
1	0.1239	0.2279	0.2927	0.3195	0.3308
2	0.0650	0.0940	0.1750	0.1842	0.1765
3	0.1533	0.1196	0.2242	0.2459	0.2584

[a] Sample 1, ice cream prepared from milk fat material A; sample 2, ice cream prepared from milk fat material B; sample 3, ice cream prepared from milk fat material A to which was added 1000 ppm tocopherol.

lowest was between flavor score and hexanal ($r^2 = 0.302$). In this study, the amount of *trans*-2-decenal contributed strongly to oxidized off-flavor of light-induced deterioration of ice cream. These results indicated that ice cream containing unsaturated FA developed aldehydes that were related to flavor deterioration. The volatile aldehydes showing a strong impact on flavor score were not derived from specific unsaturated FA.

Unsaturated FA break down through lipid oxidation to several volatile carbonyl compounds. The degree of oxidation of FA increases in the order n-9 < n-6 < n-3. Keppler (18) reported that more undesirable aldehydes are formed from n-3 unsaturated FA than from n-6 unsaturated FA. The characteristics of off-flavors derived from volatile compounds can depend on their interactions, concentration ranges, temperature, and the medium in which they are tested. A good agreement was observed in this study between an oxidized off-flavor and some volatile compounds. The origin of *trans*-2-decenal and 2,4-heptadienal are the 9-OOH hydroperoxide from oleic acid and the 12-OOH hydroperoxide from linolenic acid, respectively. Of the identified volatile carbonyl compounds arising from the autoxidative breakdown of linoleic acid at low and moderate temperatures, hexanal (66 mol%) was the major volatile (19). Hexanal was developed by breakdown from 12-/13-OOH of hydroperoxides from linolate acid and was also produced by

TABLE 2
Development of Volatile Aldehydes in Ice Cream Exposed to Fluorescent Light

Volatile aldehydes	Sample number[a]	Storage period (d) 0	1	3	5	7
		Concentration (ppm)				
Pentanal	1	0.0768	0.1302	0.1440	0.1301	0.1316
	2	0.0537	0.0510	0.0838	0.0701	0.0569
	3	0.0991	0.0555	0.1095	0.1031	0.1088
Hexanal	1	0.0044	0.0095	0.0111	0.0104	0.0120
	2	0.0056	0.0070	0.0133	0.0128	0.0121
	3	0.0065	0.0044	0.0093	0.0082	0.0097
Heptanal	1	0.0018	0.0044	0.0056	0.0058	0.0058
	2	0.0000	0.0021	0.0044	0.0042	0.0041
	3	0.0023	0.0020	0.0034	0.0032	0.0037
Octanal	1	0.0019	0.0094	0.0139	0.0174	0.0196
	2	0.0000	0.0038	0.0096	0.0105	0.0108
	3	0.0044	0.0055	0.0121	0.0131	0.0136
Nonanal	1	0.0026	0.0060	0.0072	0.0082	0.0087
	2	0.0030	0.0043	0.0060	0.0061	0.0060
	3	0.0040	0.0038	0.0060	0.0058	0.0083
t,2-Heptenal	1	0.0040	0.0059	0.0059	0.0050	0.0056
	2	0.0000	0.0018	0.0029	0.0025	0.0026
	3	0.0043	0.0034	0.0058	0.0054	0.0061
t,2-Octenal	1	0.0029	0.0060	0.0091	0.0115	0.0133
	2	0.0000	0.0023	0.0061	0.0078	0.0088
	3	0.0027	0.0031	0.0074	0.0093	0.0099
t,2-Nonenal	1	0.0018	0.0057	0.0064	0.0072	0.0087
	2	0.0000	0.0029	0.0044	0.0051	0.0054
	3	0.0022	0.0028	0.0038	0.0053	0.0055
t,2-Decenal	1	0.0248	0.0465	0.0830	0.1162	0.1173
	2	0.0027	0.0188	0.0421	0.0602	0.0653
	3	0.0255	0.0367	0.0613	0.0841	0.0855
2,4-Heptadienal	1	0.0029	0.0043	0.0065	0.0077	0.0082
	2	0.0000	0.0000	0.0024	0.0049	0.0045
	3	0.0023	0.0024	0.0056	0.0084	0.0073

[a] For sample identifications see Table 1.

TABLE 3
Correlation of the Presence of Volatile Aldehydes with Flavor Score

Volatile aldehydes	Correlation coefficients with flavor score (r^2)	Origin[a]
Pentanal	0.344	C18:2
Hexanal	0.302	C18:2
Heptanal	0.474	C18:1
Octanal	0.768	C18:1
Nonanal	0.842	C18:1
t,2-Heptenal	0.432	C18:2
t,2-Octenal	0.827	C18:2
t,2-Nonenal	0.722	C18:2
t,2-Decenal	0.864	C18:1
2,4-Heptadienal	0.851	C18:3
Total volatile aldehydes	0.365	

[a] C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid.

degradation from other unsaturated aldehydes, such as 2-octenal and 2,4-decadienal. The rates of formation were affected by the concentration of other precursors. The development of hexanal may be affected by factors particularly involved in the manufacture of ice cream, such as storage temperature, physical properties, FA composition of ingredients used (i.e., milk fat materials), or the experimental procedure. The high correlation between alde-hydes derived from oleic acid decomposition and the sensory score in this study was due to the fact that oleic acid is the major FA among milk FA, in spite of its higher oxidative stability. These results suggest that the determination of PV and total volatile aldehydes is not enough to explain light-induced flavor deterioration of ice cream, and it is more important to pay attention to several specific aldehydes.

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