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Changes in Dark Chocolate Volatiles during Storage

Lia M. Nightingale,[†] Keith R. Cadwallader,[§] and Nicki J. Engeseth*[,§]

[†]Life Sciences Division, Palmer College of Chiropractic, Davenport, Iowa 52803, United States

[§]Department of Food Science and Human Nutrition, University of Illinois, Urbana, Illinois 61801, United States

ABSTRACT: Chocolate storage is critical to the quality of the final product. Inadequate storage, especially with temperature fluctuations, may lead to a change in crystal structure, which may eventually cause fat bloom. Bloom is the main cause of quality loss in the chocolate industry. The impact of various storage conditions on the flavor quality of dark chocolate was determined. Dark chocolate was stored in different conditions leading to either fat or sugar bloom and analyzed at 0, 4, and 8 weeks of storage. Changes in chocolate flavor were determined by volatile analysis and descriptive sensory evaluation. Results were analyzed by analysis of variance (ANOVA), cluster analysis, principal component analysis (PCA), and linear partial least-squares regression analysis (PLS). Volatile concentration and loss were significantly affected by storage conditions. Chocolates stored at high temperature were the most visually and texturally compromised, but volatile concentrations were affected the least, whereas samples stored at ambient, frozen, and high relative humidity conditions had significant volatile loss during storage. It was determined that high-temperature storage caused a change in crystal state due to the polymorphic shift to form VI, leading to an increase in sample hardness. Decreased solid fat content (SFC) during high-temperature storage increased instrumentally determined volatile retention, although no difference was detected in chocolate flavor during sensory analysis, possibly due to instrumental and sensory sampling techniques. When all instrumental and sensory data had been taken into account, the storage condition that had the least impact on texture, surface roughness, grain size, lipid polymorphism, fat bloom formation, volatile concentrations, and sensory attributes was storage at constant temperature and 75% relative humidity.

KEYWORDS: *chocolate, bloom, polymorphism, flavor, volatile*

INTRODUCTION

The unique chocolate matrix is a mixture of sugar and cocoa solids dispersed in a cocoa butter phase, yet its specific packing structure and particle interactions make chocolate an even more intriguing and complex substance. Characteristics of chocolate texture are due to both the ratio of solid to liquid fat in a product (solid fat content, SFC) and the crystal state the solid portion is found in, known as the lipid polymorph. A higher SFC affects hardness, melting, and sweetness perception.[1]

Chocolate flavor is a combination of volatile compounds for aroma, water-soluble compounds for taste, and physical interactions for mouthfeel.[2] Kinsella[3] observed that component volatility largely depends on concentration in the vapor phase, which is influenced by the rate of retention from chocolate and is dependent on temperature, molecular interactions, and partition coefficient of the particular compound. Because chocolate is a continuous lipid phase, most flavor perception occurs due to retronasal action of volatile compounds released during melting.[4] Structural changes of the lipid phase may alter volatile release, thus changing the flavor profile of the chocolate. Most volatile compounds in chocolate are formed via nonenzymatic browning during processing. The most potent compounds related to chocolate flavor are the Strecker aldehydes and pyrazines, although the overall flavor profile is a combination of many components, including several sulfur compounds.[5]

Chocolate has a shelf life of approximately 12−24 months.[6,7] As chocolate is stored, structural changes occur. Various storage conditions may lead to the development of either fat bloom or sugar bloom, each of which compromises both visual and textural quality. Bloom is the main cause of quality loss in the chocolate industry.[8] With total chocolate sales nearly $15 billion annually in the United States, loss due to bloom formation may be substantial.[9] Market loss due to fat bloom is difficult to verify, because these changes arise many months after processing and often occur many steps down the distribution ladder. As bloom forms, solid chocolate particle size may also increase, but whether microstructural and perceptual changes also occur and their impact on volatile release are unclear. Despite the importance of correlating sensory results with instrumental texture and flavor properties in stored chocolate, such studies are nonexistent.

MATERIALS AND METHODS

Materials. Dove Dark Chocolate (Masterfoods, Inc., Hacketts-town, NJ, USA; 32.5% cocoa butter w/w; 47.5% sugar w/w) was acquired from a local grocer and stored immediately without wrappers for up to 8 weeks in the following conditions: ambient storage room [23.0 °C and 45.4% relative humidity (RH)], ambient 57% RH (23.0 °C and 57.6% RH), ambient 75% RH (23.0 °C and 75.3% RH), freezer (−27.2 °C and 40.9% RH), high temperature (30.5 °C and 44.1% RH), and high temperature with fluctuations (30.5 ± 1.7 °C and 77.0% RH). Samples purchased at the same time were from the same lots. Incubator temperatures fluctuated every 3 h. Relative humidity values were obtained using a Thermo hygrometer Humidity Measuring Stick (Cole-Parmer Instrument Co., Vernon Hills, IL, USA) and may be approximate. Salt solutions were used to attain the

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Table 1. Calibration Data Used for Quantification of Selected Volatile Components of Dark Chocolate by Dynamic Headspace Analysis/Gas Chromatography/Mass Spectroscopy

standard	compound	concentration[a] (ng/g)	RI[b]	m/z[c]	slope	Y-intercept	R^2 [d]
1	2-methylpropanal	3.94−7880	806	72	219832786.77	−4215473.92	0.99
2	2(3)-methylbutanal	10.62−21240	911	58	92065383.13	55454753.43	0.99
3	dimethyl disulfide	2.70−5400	1057	94	141034426.91	564712.37	0.99
4	hexanal	2.24−4480	1068	44	50007498.87	2819821.16	0.99
5	myrcene	1.96−3920	1147	93	13826759.10	−113287.19	0.99
6	limonene	3.42−6840	1184	68	15168649.56x	18988527.19	0.98
7	methyl pyrazine	8.12−16240	1259	94	73411537.65	−2593506.40	0.99
8	2,5(6)-dimethylpyrazine	12.88−25760	1316	108	45045895.19	−9557348.59	0.99
9	2,3-dimethylpyrazine	2.98−5960	1341	108	52077124.23	1440638.96	0.99
10	dimethyl trisulfide	2.10−4200	1357	126	31507055.00	−660579.87	0.99
11	2-nonanone	2.54−5080	1370	58	20129964.69	263388.67	0.99
12	2-ethyl-5(6)-methylpyrazine	2.88−5760	1373	121	20587910.53	−995011.71	0.99
13	2-ethyl-3-methylpyrazine	3.24−6480	1393	121	21703615.00	666736.77	0.99
14	trimethylpyrazine	4.56−9120	1395	122	17181690.12	140341.64	0.99
15	1-octen-3-ol	3.48−6960	1416	57	34886336.20	2795993.56	0.99
16	2(3)-ethyl-2(3),5-dimethylpyrazine	7.16−14320	1440	135	13664643.23	211670.37	0.99
17	2-furfural	2.66−5320	1445	96	43526548.02	2114051.26	0.99
18	tetramethylpyrazine	1.98−3960	1463	54	12473176.61	106669.67	0.99
19	propanoic acid	7.32−14640	1496	74	4523739.31	4466466.71	0.99
20	linalool	2.04−4080	1514	71	4136452.78	5176.3	0.99
21	2-methylpropanoic acid	24.82−49640	1522	73	8346974.22	7274233.6	0.99
22	butyric acid	21.22−42440	1595	60	10993961.30	−735567.30	0.99
23	3-methylbutyric acid	21.36−42720	1626	60	18939536.67	2172088.84	0.99
24	2-methoxyphenol	6.48−12960	1831	109	6447847.73	−635529.07	0.99
25	acetylpyrrole	2.00−4000	1939	94	3801100.23	−1069161.31	0.99

[a]Actual concentration range used for standard curve construction. [b]RI, retention index on Stabilwax DA column. [c]Mass ion used for identification of selected volatile. [d]Coefficient of determination of standard curves used for quantification of volatile compounds.

high RH conditions. Sodium bromide was purchased from Leisure Time (Alpharetta, GA, USA), and sodium chloride was obtained from Fisher Scientific Co. (Fair Lawn, NJ, USA) for use in relative humidity chambers. All solvents were purchased from Fisher Scientific Co. Deodorized cocoa butter was purchased from Cedar Vale Natural Health (Cedar Vale, KS, USA). Volatile standards 1−14 and 16−25 listed in Table 1 were purchased from Sigma-Aldrich, Co. (St. Louis, MO, USA), whereas standard 15 was purchased from Bedoukian Research Inc. (Danbury, CT, USA).

Analysis of Volatile Compounds. Volatile compounds in fresh and stored chocolate were analyzed by dynamic headspace analysis/gas chromatography/mass spectroscopy (DHA/GC/MS). Five grams of finely chopped chocolate was weighed into a Tekmar 3000 purge-and-trap concentrator (Tekmar Inc., Cincinnati, OH, USA), which was preheated at 40 °C, and mixed for 10 min to completely melt the sample. The sample was then flushed with nitrogen at a flow rate of 50 mL/min for 25 min at 40 °C with mixing as the volatiles were purged onto a Tenax TA trap (no. 1; Tekmar Inc.). The trap was dry-purged for 5 min to remove moisture and then thermally transferred at 200 °C for 8 min. Volatiles were cryofocused at −120 °C onto a deactivated fused silica capillary column (15 cm × 0.53 mm i.d.). Trap pressure control was set at 4 psi during purging. Helium flow through the Tenax trap (20 mL/min) and cryofocusing trap (1.3 mL/min) was controlled by the GC split/splitless pressure control pneumatics. Compounds were desorbed (200 °C for 1 min) directly onto a fused silica GC column (Stabilwax DA, 30 m × 0.25 mm i.d. × 0.50 μm film thickness; Restek Corp., Bellefonte, PA, USA). Between runs, the system was cleaned by running a blank by purging clean glassware and the Tenax trap was baked at 220 °C for 10 min to remove residual volatiles.

GC-MS analysis was conducted using a HP 6890 GC/5973 mass selective detector (MSD; Agilent Technologies, Inc., Palo Alto, CA, USA). The GC oven temperature was programmed to increase from 35 to 225 °C at a rate of 4 °C/min, with initial and final hold times of 5 and 20 min, respectively. The MSD conditions were as follows: ion source temperature, 230 °C; ionization energy, 70 eV; mass scan range, 35−300 amu; electron multiplier voltage, 2900 V; scan rate, 4.5 s^{-1}; and ion mode, electron ionization (EI). All analyses were performed in triplicate for each chocolate sample, and results were averaged.

Compound Identification and Quantification. An odorless chocolate matrix was constructed by removing volatiles from unsweetened baking chocolate (Kraft Foods North America, Inc., Rye Brook, NY, USA) by Soxhlet extraction with ether for 4 h followed by extraction with 96% (v/v) ethanol for 7 h. The odorless cocoa was allowed to dry before the addition of 36 g to 64 g of deodorized cocoa butter (Cedar Vale Natural Health) to make 100 g of odorless chocolate matrix. Compounds were positively identified by comparing retention indices and mass spectra of unknowns to those of authentic standard compounds added to an odorless chocolate matrix and analyzed under identical conditions. Relevant compounds were quantified using external calibration. A super stock solution containing all 25 authentic standards was constructed, diluted to 1:10 and 1:100, and added to the odorless chocolate matrix in volumes ranging from 0.5 to 20 μL. External calibration was utilized to take into account any residual volatiles that may have remained in the odorless chocolate matrix. Residual volatiles in deodorized cocoa butter were taken into account in construction of the standard curves. Table 1 lists the volatile compounds quantified, retention indices, mass ions, concentration ranges used for calibration, y-intercepts, and slopes of the calibration curve. Retention indices were calculated on the basis of retention times of the standard n-alkanes.[10,11]

Sensory Evaluation. Ten panelists (3 males and 7 females, aged 18−39 years) were recruited and trained in the technique of descriptive analysis. Judges were trained for 20 h over 4 weeks. Panelists were trained to analyze chocolate texture and flavor using 15 cm line scales with word anchors and time−intensity measurements for melting time. Rinsing protocol, references, and terms were generated by the panel. Texture attributes generated by the panel included hardness, cohesiveness, chewiness, fatty mouthcoating, dry

Table 2. Effect of Dark Chocolate Storage Conditions on Flavor Volatile Concentrations Assessed by Dynamic Headspace Analysis/Gas Chromatography/Mass Spectroscopy[a]

	concentration (ng/g)				
storage conditions[b]	2-methylpropanal	2(3)-methylbutanal	dimethyl disulfide	hexanal	myrcene
0 weeks	52.1 ± 6.4 gh	315 ± 46 f	14.7 ± 1.3 c	69.6 ± 15.4 de	472 ± 59 de
4 weeks, amb	33.6 ± 1.0 ij	132 ± 6 i	3.80 ± 0.10 hij	26.7 ± 1.9 f	18.3 ± 1.5 h
8 weeks, amb	31.8 ± 2.6 j	79.0 ± 15.2 i	2.80 ± 0.40 j	47.3 ± 14.2 ef	10.6 ± 0.5 h
4 weeks, fre	58.5 ± 3.0 fg	289 ± 14 fg	5.90 ± 0.10 fg	33.8 ± 7.1 f	60.4 ± 0.6 gh
8 weeks, fre	44.5 ± 2.6 hi	207 ± 8 h	8.10 ± 0.20 e	30.1 ± 5.3 f	93.1 ± 5.1 g
4 weeks, 30.5 °C	83.8 ± 8.0 d	499 ± 43 d	9.7 ± 0.6 d	46.0 ± 0.9 ef	455 ± 2 e
8 weeks, 30.5 °C	78.9 ± 1.8 de	606 ± 8 c	15.3 ± 0.1 c	41.0 ± 1.8 ef	564 ± 12 c
4 weeks, 30.5 ± 1.7 °C	100 ± 2 c	640 ± 25 c	10.4 ± 0.8 d	37.3 ± 0.6 ef	518 ± 30 cd
8 weeks, 30.5 ± 1.7 °C	69.4 ± 1.0 ef	410 ± 10 e	9.6 ± 0.4 d	148 ± 18 c	270 ± 6 f
4 weeks, 57% RH	59.6 ± 1.9 fg	246 ± 12 gh	4.5 ± 0.1 ghi	30.8 ± 0.6 f	29.0 ± 0.3 h
8 weeks, 57% RH	32.4 ± 1.6 j	71.1 ± 15.8 i	3.1 ± 0.2 ij	18.2 ± 2.2 f	22.0 ± 3.4 h
4 weeks, 75% RH	81.0 ± 7.6 d	428 ± 22 e	6.4 ± 0.3 f	90.0 ± 25.7 d	40.0 ± 1.1 gh
8 weeks, 75% RH	65.0 ± 1.8 f	251 ± 6 gh	4.7 ± 0.2 gh	17.2 ± 2.0 f	31.4 ± 1.7 h

	concentration (ng/g)				
storage conditions	limonene	methylpyrazine	2,5(6)-dimethylpyrazine	2,3-dimethylpyrazine	dimethyl trisulfide
0 weeks	10700 ± 564 c	173 ± 14 d	643 ± 47 c	90.9 ± 7.4 d	50.8 ± 3.9 e
4 weeks, amb	341 ± 13 kl	95.2 ± 2.6 h	215 ± 8 ij	13.5 ± 0.5 hi	10.9 ± 0.5 hi
8 weeks, amb	148 ± 23 l	56.5 ± 2.8 i	192 ± 7 j	5.3 ± 1.1 j	9.3 ± 0.4 i
4 weeks, fre	1580 ± 33 h	111 ± 2 fg	271 ± 4 gh	27.6 ± 0.5 g	16.1 ± 1.5 gh
8 weeks, fre	2440 ± 31 g	118 ± 2 f	361 ± 7 f	40.4 ± 0.8 f	19.6 ± 0.2 g
4 weeks, 30.5 °C	8336 ± 43 e	147 ± 2 e	483 ± 4 d	71.4 ± 0.2 e	61.5 ± 0.4 d
8 weeks, 30.5 °C	9080 ± 47 d	196 ± 2 c	627 ± 2 c	106 ± 1 c	78.3 ± 1.5 c
4 weeks, 30.5 ± 1.7 °C	8800 ± 149 de	185 ± 7 cd	619 ± 18 c	97.4 ± 4.2 d	80.2 ± 2.5 c
8 weeks, 30.5 ± 1.7 °C	6610 ± 80 f	124 ± 2 f	435 ± 9 e	71.3 ± 1.7 e	35.5 ± 1.0 f
4 weeks, 57% RH	836 ± 3 ij	117 ± 1 f	255 ± 1 ghi	19.5 ± 0.6 h	15.4 ± 0.2 gh
8 weeks, 57% RH	650 ± 16 jk	66.0 ± 2.7 i	197 ± 3 j	11.8 ± 0.7 ij	12.0 ± 0.3 hi
4 weeks, 75% RH	1290 ± 1 hi	142 ± 1 e	298 ± 2 g	28.2 ± 0.6 g	14.2 ± 4.0 hi
8 weeks, 75% RH	885 ± 10 ij	101 ± 2 gh	240 ± 2 hi	18.1 ± 0.1 hi	12.0 ± 0.6 hi

	concentration (ng/g)				
storage conditions	2-nonanone	2-ethyl-5(6)-methylpyrazine	2-ethyl-3-methylpyrazine	trimethylpyrazine	1-octen-3-ol
0 weeks	442 ± 30 c	163 ± 16 d	32.1 ± 2.1 d	370 ± 22 d	62.9 ± 5.3 cd
4 weeks, amb	73.6 ± 5.8 j	18.7 ± 0.5 fg	nd	nd	21.0 ± 1.3 h
8 weeks, amb	96.1 ± 16.5 ij	9.7 ± 0.0 g	nd	nd	21.4 ± 2.4 h
4 weeks, fre	171 ± 6 g	31.6 ± 1.8 fg	10.6 ± 5.4 efg	28.8 ± 49.9 gh	26.2 ± 0.5 gh
8 weeks, fre	253 ± 8 f	51.5 ± 0.3 f	10.1 ± 0.8 efg	nd	33.1 ± 2.7 fg
4 weeks, 30.5 °C	309 ± 2 e	140 ± 4 de	25.8 ± 0.7 de	311 ± 5 e	54.1 ± 0.9 e
8 weeks, 30.5 °C	393 ± 7 d	213 ± 1 c	52.1 ± 11.1 c	431 ± 14 c	70.5 ± 1.3 c
4 weeks, 30.5 ± 1.7 °C	378 ± 10 d	234 ± 39 c	62.2 ± 18.1 c	426 ± 50 c	66.4 ± 2.3 c
8 weeks, 30.5 ± 1.7 °C	291 ± 17 e	109 ± 1 e	24.3 ± 1.1 def	286 ± 6 cq	56.7 ± 2.8 de
4 weeks, 57% RH	121 ± 2 h	31.3 ± 1.2 fg	nd	68.3 ± 1.7 fg	26.1 ± 2.3 gh
8 weeks, 57% RH	187 ± 5 g	24.6 ± 1.8 fg	nd	nd	23.7 ± 7.5 h
4 weeks, 75% RH	195 ± 2 g	46.5 ± 0.7 f	7.5 ± 0.5 fg	90.5 ± 0.9 f	36.5 ± 2.2 f
8 weeks, 75% RH	198 ± 2 g	35.5 ± 0.2 fg	nd	nd	38.4 ± 1.3 f

	concentration (ng/g)				
storage conditions	2(3)-ethyl-2(3),5-dimethylpyrazine	2-furfural	tetramethylpyrazine	propanoic acid	linalool
0 weeks	560 ± 31 cd	42.1 ± 3.3 f	222 ± 15 cd	1120 ± 60 e	1660 ± 122 c
4 weeks, amb	27.0 ± 6.5 i	42.3 ± 0.4 f	105.4 ± 3.7 h	818 ± 35 gh	252 ± 12 h
8 weeks, amb	nd	21.1 ± 2.0 h	106 ± 7 h	769 ± 55 h	184 ± 9 h
4 weeks, fre	101 ± 13 h	38.8 ± 0.9 f	120 ± 1 gh	641 ± 17 i	473 ± 12 g
8 weeks, fre	161 ± 8 g	38.4 ± 0.3 f	139 ± 8 fg	931 ± 14 f	586 ± 26 ef
4 weeks, 30.5 °C	432 ± 12 e	57.0 ± 3.7 d	198 ± 2 e	870 ± 20 fg	1370 ± 7 d
8 weeks, 30.5 °C	576 ± 1 c	77.5 ± 2.1 c	244 ± 4 c	1800 ± 35 c	1590 ± 30 c
4 weeks, 30.5 ± 1.7 °C	531 ± 16 e	71.9 ± 2.3 cd	232 ± 7 c	1080 ± 36 e	1630 ± 45 c
8 weeks. 30.5 ± 1.7 °C	386 ± 11 f	40.5 ± 0.9 f	205 ± 8 de	863 ± 4 fgh	1310 ± 34 d
4 weeks, 57% RH	107 ± 4 h	56.9 ± 0.2 d	120 ± 6 gh	1100 ± 8 e	558 ± 7 efg
8 weeks, 57% RH	84.2 ± 0.4 h	31.7 ± 2.9 g	113 ± 21 h	816 ± 45 gh	480 ± 8 fg
4 weeks, 75% RH	143 ± 3 g	69.4 ± 0.7 d	149 ± 6 f	1320 ± 9 d	648 ± 2 e

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Table 2. continued

storage conditions	concentration (ng/g)				
	2(3)-ethyl-2(3),5-dimethylpyrazine	2-furfural	tetramethylpyrazine	propanoic acid	linalool
8 weeks, 75% RH	97.7 ± 3.8 h	52.9 ± 1.6 e	154 ± 4 f	1370 ± 32 d	513 ± 9 fg
storage conditions	concentration (ng/g)				
	2-methylpropanoic acid	butyric acid	3-methylbutyric acid	2-methoxyphenol	acetylpyrrole
0 weeks	1820 ± 114 e	973 ± 35 d	3440 ± 150 e	679 ± 45 c	902 ± 56 c
4 weeks, amb	1090 ± 32 g	590 ± 5 ghi	2050 ± 48 i	94.5 ± 9.5 h	381 ± 6 h
8 weeks, amb	835 ± 69 h	607 ± 38 gh	1520 ± 104 j	101 ± 4 h	321 ± 16 h
4 weeks, fre	1100 ± 22 g	518 ± 21 i	2210 ± 35 i	130 ± 2 gh	431 ± 18 h
8 weeks, fre	1430 ± 20 f	642 ± 69 fg	2600 ± 87 h	215 ± 5 f	550 ± 17 g
4 weeks, 30.5 °C	1710 ± 16 e	963 ± 13 d	3310 ± 18 ef	288 ± 13 e	665 ± 6 ef
8 weeks, 30.5 °C	2670 ± 49 c	1260 ± 16 c	4040 ± 63 c	368 ± 8 d	873 ± 8 c
4 weeks, 30.5 ± 1.7 °C	2070 ± 55 d	851 ± 16 e	3700 ± 82 d	321 ± 3 e	782 ± 20 d
8 weeks, 30.5 ± 1.7 °C	1760 ± 21 e	659 ± 11 fg	3170 ± 46 f	316 ± 5 e	700 ± 5 e
4 weeks, 57% RH	1520 ± 10 f	695 ± 12 f	2800 ± 18 g	146 ± 2 g	621 ± 6 f
8 weeks, 57% RH	1140 ± 41 g	555 ± 23 hi	2230 ± 53 i	128 ± 1 gh	502 ± 5 g
4 weeks, 75% RH	1850 ± 13 e	656 ± 6 fg	3190 ± 48 f	155 ± 5 g	660 ± 6 ef
8 weeks, 75% RH	1720 ± 15 e	655 ± 2 fg	2920 ± 16 g	122 ± 3 gh	553 ± 10 g

[a]Results stated as the mean ± SEM (n = 3). Results with same letters (c–k) were not significantly different at $p \leq 0.05$. [b]0, 4, and 8 weeks indicate storage length in assigned condition. Abbreviations: amb, ambient conditions; fre, freezer; 30.5 °C, heating chamber set at 30.5 °C; 30.5 ± 1.7°C, heating chamber with fluctuating temperature; 57% RH, chamber set to 57% relative humidity; 75% RH, chamber set to 75% relative humidity.

mouthfeel, and toothpacking and were evaluated after expectoration. For flavor, terms established by the panel included sweetness, bitterness, chocolate flavor, cream flavor, and roasted aftertaste. Judges analyzed all samples in duplicate, tasting five to six samples per session. Compusense Five 4.2 software (Compusense, Inc., Ontario, Canada) was used for data collection. Samples were served in 2 oz plastic cups with lids and labeled with random three-digit numbers, evaluated under black light at ambient temperature and relative humidity. Time–intensity measurements of chocolate melting time were analyzed by placing a previously measured sample (0.25–0.30 g) between the tongue and the roof of the mouth and rating over 2 min or until the sample was completely melted.

Statistical Analysis. Data were analyzed using Statistical Analysis Software (SAS) v. 9.1 (SAS Institute Inc., Cary, NC) to determine the analysis of variance (ANOVA) and Fisher's least significant difference (LSD) for all results. Mean ratings of significant attributes ($p < 0.05$) were further analyzed using covariance matrices for principal component analysis (PCA) in SAS. Multivariate statistical analysis was performed by linear partial least-squares regression analysis (PLS) using Unscrambler software (CAMO Technologies Inc., Woodbridge, NJ, USA). PLS is a technique combining features from principal component analysis and multiple linear regression, in which one would predict or analyze a set of dependent (response) variables from a set of predictors.[12] In this PLS analysis, sensory attributes served as the response variables (Y) and instrumental measurements serve as the predictor variables (X). PLS2 was used to correlate all statistically significant instrumental measurements and sensory attributes, whereas relationships between instrumental texture data and a single sensory attribute were evaluated by PLS1.

RESULTS

Chocolate flavor perception may be affected by aroma compound volatility, which is associated with polymorph transition and solid fat content. Volatiles quantified in Dove Dark Chocolate are listed in Table 1, whereas final concentrations are presented in Table 2. All 25 volatiles quantified in dark chocolate were significantly ($p < 0.05$) affected by storage. Strecker aldehydes are responsible for the chocolate/malty characteristics of chocolate.[13] The main aldehydes contributing to chocolate flavor are 2-methylpropanal and 2(or 3)-methylbutanal. Chocolate stored at high temperature (30.5 °C) with or without fluctuations had significantly higher concentrations of 2-methylpropanal and 2- and 3-methylbutanal. Storage at ambient conditions (23.0 °C) and high relative humidity (75%) significantly decreased 2-methylpropanal, 2(or 3)-methylbutanal, and 2-furfural (caramel-like, sweet).[14] Volatiles decreased more rapidly at 57% RH than at 75% RH.

Pyrazines are responsible for the cocoa-like, roasted aroma of chocolate.[13−15] In general, storage at high temperature without fluctuations for up to 8 weeks or for 4 weeks with temperature fluctuations did not significantly affect the concentrations for most pyrazines. However, several pyrazines were affected by storage condition. Storage at ambient and high relative humidity conditions caused 2,3-dimethylpyrazine (hazelnut, roasted),[13] 2-ethyl-5(or)6-methylpyrazine (cocoa, roasted),[13,14] 2-ethyl-3-methylpyrazine (cocoa, roasted),[14,17] trimethylpyrazine (cocoa, roasted),[13] and 2(or 3)-ethyl-2(or 3),5-dimethylpyrazine (cocoa, roasted, praline)[13,15,16] to be significantly lower. Chocolate stored for up to 8 weeks at high temperature without fluctuations had significantly higher concentrations of methylpyrazine and 2,3-dimethylpyrazine. Storage at high temperature without fluctuations for up to 8 weeks and with fluctuations for 4 weeks caused significant increases in 2-ethyl-5(or 6)-methylpyrazine, 2-ethyl-3-methylpyrazine, and trimethylpyrazine.

Volatile fatty acids are formed during bean fermentation and are also important to chocolate flavor by imparting sour, buttery, and sweaty notes.[6,18,19] Storage at high temperature with and without fluctuations caused the least amount of change, whereas 8 weeks at high temperature without fluctuations caused a slight, yet significant ($p < 0.05$) increase in all volatile fatty acids: propanoic acid (rancid),[19] 2-methylpropanoic acid (butter),[19] butyric acid (cheesy),[19] and 3-methylbutyric acid (sweaty).[19] Storage at ambient and 57% relative humidity significantly decreased ($p < 0.05$) all acids quantified. Chocolate stored at 75% relative humidity had significantly less butyric acid and 3-methylbutyric acid and significantly more propanoic acid.

Finally, other volatile compounds including sulfides, alcohols, and ketones may make a significant contribution to chocolate flavor. Concentrations of sulfur compounds [dimethyl disulfide (meaty)[14] and dimethyl trisulfide (sulfury, cabbage)],[19] as well as limonene, linalool (floral)[14] myrcene, 2-methoxyphenol, 2-nonanone, and 1-octen-3-ol (mushroom)[19] were significantly decreased following storage in ambient, frozen, and high relative humidity conditions (−27.2 to 23.0 °C). Acetylpyrrole (herbal medicine)[19] significantly decreased during storage at ambient and frozen conditions but was relatively unaffected by storage at high relative humidity.

The impact of storage on volatiles and the resulting impact on sensory parameters are depicted in various ways below (Figures 1−3). Figure 1 represents the impact of storage on

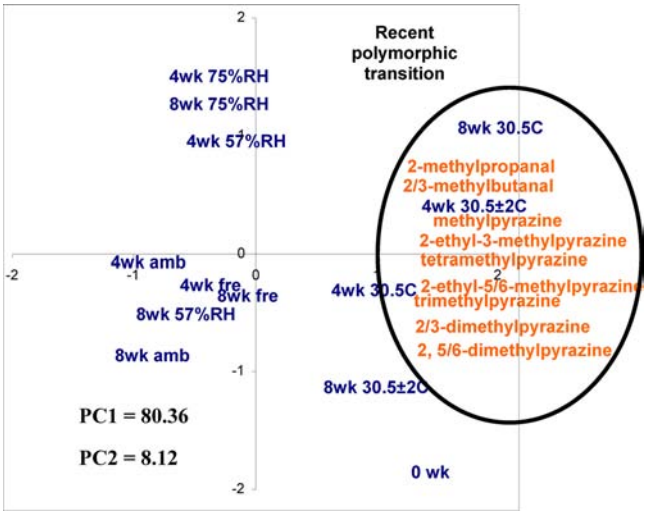

Figure 1. Principal component analysis biplot of volatile aldehydes and pyrazines quantified in dark chocolate stored in various conditions (PC1 vs PC2). 0, 4, and 8 wk indicate storage length in assigned condition. Abbreviations: amb, ambient conditions; fre, freezer; 30.5C, heating chamber set at 30.5 °C; 30.5 ± 2C, heating chamber with fluctuating temperature; 57%RH, chamber set to 57% relative humidity; 75%RH, chamber set to 75% relative humidity.

volatile aldehydes and pyrazines, as representative of the total flavor volatiles (PCA plot). Figure 2 is a representation of the correlation between sensory attributes and flavor volatiles under different storage conditions. Figure 3 illustrates the relationship between flavor volatiles and sensory attributes.

Volatile components were significantly affected by storage conditions, as illustrated by the PCA biplot in Figure 1. For easier reading, only chocolate aldehydes and pyrazines are depicted, although all volatile compounds were highly correlated (data not shown). According to the PCA plot (Figure 1), the space explained >88% of the variance in two factors and showed that dark chocolate stored at high temperature with and without fluctuations had the highest concentrations of volatile components and was the closest in volatile profile to fresh chocolate. Overall, storage at ambient conditions (23.0 °C) had the most significantly negative impact on chocolate flavor volatiles, followed by storage in the freezer and at high relative humidity. Storage at high temperature with and without fluctuations caused the least apparent volatile loss.

Sensory results were previously discussed.[20] Briefly, toothpacking, hardness, and melting time were negatively correlated with chewy, cohesive, dry mouthfeel, sweet taste, and cream

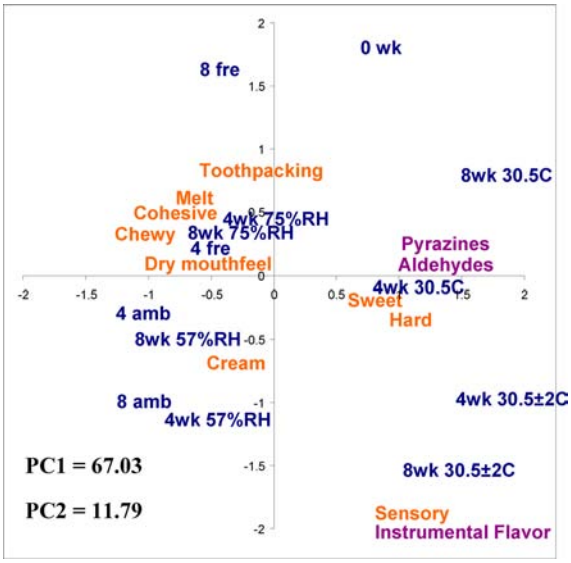

Figure 2. Principal component analysis biplot of sensory attributes and volatile aldehydes and pyrazines quantified in dark chocolate stored in various conditions (PC1 vs PC2). 0, 4, and 8 wk indicate storage length in assigned condition. Abbreviations; amb, ambient conditions; fre, freezer; 30.5C, heating chamber set at 30.5 °C; 30.5 ± 2C, heating chamber with fluctuating temperature; 57%RH, chamber set to 57% relative humidity; 75%RH, chamber set to 75% relative humidity.

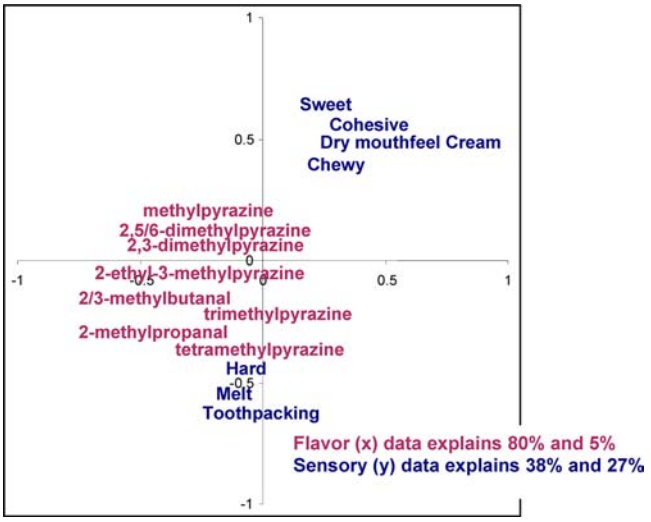

Figure 3. Linear partial least-squares regression analysis (PLS) biplot of sensory attributes versus volatile compounds quantified by dynamic headspace analysis/gas chromatography/mass spectroscopy.

flavor. Chocolate stored at high temperature with and without fluctuations was harder, had greater toothpacking, and took longer to melt. Chocolate stored without fluctuations had greater toothpacking than chocolate with temperature fluctuations. Fresh chocolate and chocolate stored at ambient, frozen, and high relative humidity conditions were more cohesive, chewier, and sweeter and had higher intensities of dry mouthfeel and cream flavor.

Sensory and Instrumental Flavor Correlation. Volatile compound changes were highly correlated with storage at high temperature with and without fluctuations. The PCA biplot (Figure 2) accounts for 78% of variance in two factors. According to this plot, chocolate volatiles positively correlated

with sensory hardness and sweet taste and negatively correlated with cohesiveness, chewiness, toothpacking, dry mouthfeel, and melting time. The negative correlation with melting time may be because during volatile analysis chocolate samples are completely melted at 40 °C, several degrees above the highest melting point prior to purging. The PLS2 biplot in Figure 3 explains 85% of the flavor volatile data and only 65% of sensory results. Sensory hardness, melting, and toothpacking were more closely correlated to all of the volatiles analyzed. Samples that were harder took longer to melt during sensory analysis; these samples also had the highest levels of flavor volatiles. Similarly, PLS1 factor loadings (Table 3) determined that sensory

Table 3. Percent Variance of Sensory Attributes (y Variable) of All Samples As Explained by Instrumental Texture Measurements (x Variable) As Indicated[a]

sensory modality/attribute	texture variance % y-explained (PC1, PC2)	volatiles variance % y-explained (PC1, PC2)
all attributes	65, 7	38, 27
texture		
hardness	81, 8	54, 23
cohesivness	84, 5	45, 36
chewiness	78, 0	60, 21
dry mouthfeel	63, 7	13, 36
toothpacking	47, 18	23, 40
melting	86, 4	48, 37
flavor		
sweet	65, 5	24, 49
cream	69, 9	46, 26

[a]Total variance was accounted for by first two PLS factors.

hardness, cohesiveness, chewiness, melting time, sweet taste, and cream flavor were explained by chocolate volatile results.

DISCUSSION

Aroma intensity during consumption of a food product is a combination of the concentration of the aroma volatiles present and their ability to reach the sensory system in an allotted time. The ability of these compounds to be released from the food product is a function of the product composition, texture, and ratio of solid to liquid components. Previously, the impact of various storage conditions, textural changes, and polymorphic transitions of chocolate on volatile concentration and release had not been determined.

Storage condition significantly affected all volatiles analyzed in this experiment. In general, storage of chocolate at ambient, frozen, and high relative humidity conditions (−27.2 to 23.0 °C) resulted in a significant decrease in volatiles, possibly due to evaporation or oxidative reactions. Although this was not expected, volatile loss during frozen storage has been previously reported.[21,22] Storage at high temperature with and without fluctuations caused little change in volatile loss or a significant increase in some volatiles as compared to fresh chocolate, possibly due to Maillard reactions. Volatile concentrations were less in chocolate that had transitioned to polymorph VI at 4 weeks and was stored for an additional 4 weeks (8 weeks at 30.5 ± 1.7 °C), signifying that prolonged storage past the polymorphic transition to form VI may exacerbate chocolate volatile loss.

Other instrumental and sensory results have been previously reported for these samples.[20] Instrumental analyses included texture analyzer, color analysis, X-ray diffraction (XRD), atomic force microscopy (AFM), differential scanning calorimetry (DSC), and triglyceride analysis. Table 4 lists the melting point

Table 4. Average Chocolate Melting Point Determined by Differential Scanning Calorimetry and Polymorphic Forms Determined by X-ray Diffraction[a]

storage conditions	melting point[b](°C)	polymorph
0 weeks	33.5 ± 0.5 fg	V
4 weeks, amb	34.0 ± 0.0 ef	V
8 weeks, amb	33.7 ± 0.2 fg	V
4 weeks, fre	34.4 ± 0.3 e	V
8 weeks, fre	33.4 ± 0.1 g	V
4 weeks, 30.5 °C	35.4 ± 0.0 d	V
8 weeks, 30.5 °C	35.8 ± 0.2 cd	VI
4 weeks, 30.5 ± 1.7 °C	36.3 ± 0.1 c	VI
8 weeks, 30.5 ± 1.7 °C	36.1 ± 0.4 c	VI
4 weeks, 57% RH	33.7 ± 0.2 fg	V
8 weeks, 57% RH	33.5 ± 0.2 g	V
4 weeks, 75% RH	33.5 ± 0.2 fg	V
8 weeks, 75% RH	33.9 ± 0.3 efg	V

[a]Data were previously reported in ref 20. [b]Results are stated as the mean ±SD. Numbers with same letters (c−g) were not significantly different at $p \leq 0.05$.

and polymorphic form for each chocolate sample. It was determined that chocolate samples stored for 4 weeks at high temperature with fluctuations or for 8 weeks without fluctuations had transitioned from polymorph V to VI. The polymorphic transition that occurred in these samples affected the chocolate melting point and may have influenced SFC, which in turn may prevent flavor volatile loss surrounding the polymorphic transition from form V to VI. Fresh chocolate had a melting point of 33.5 °C, whereas chocolate that had transitioned to polymorph VI had a melting point of 35.8 °C.[20] Continued storage of chocolate following the polymorphic transition to form VI (8 weeks with fluctuations) caused chocolate volatiles to decrease, but at a much slower rate than other storage conditions (ambient, frozen, and relative humidity). Although high-temperature storage without fluctuations elicited a polymorphic transition to form VI following 8 weeks of storage, samples were not visually bloomed.[20] Chocolate stored at high temperature with fluctuations contained significant amounts of fat bloom, with longer storage facilitating more fat bloom formation. Therefore, the rate of volatile release during storage may be a combination of length of time past the polymorphic transition and the amount of visual fat bloom formed.

Sample hardness may also affect the concentration and release of volatile compounds. According to recent research, the hardness of gels has been associated with an increase in volatile concentration and also with a decrease in the rate of volatile release.[23,24] On the basis of the current study, sample hardness was increased following high-temperature storage, which also correlated with amplified volatile concentrations for compounds associated with chocolate flavor. Gierczynski et al.[24] indicated that sample breakage, leading to changes in food structure, would have a significant impact on aroma release. Fresh samples and chocolate stored at ambient, frozen, and high relative humidity conditions would be much more homogeneous than chocolate that had transitioned to polymorph VI and formed fat bloom (i.e., high-temperature

storage). These bloomed, heterogeneous samples (high-temperature storage with and without fluctuations) have increased sample hardness; similar to the in vitro data of Gierczynski et al.,[24] they may have impaired volatile release. It is speculated that impaired volatile release may be due to compositional rearrangement associated with a polymorphic transition, although when completely melted during flavor volatile analysis, they had higher concentrations of these volatiles. Our results also indicate that volatile concentrations increased through the polymorphic transition to form VI; extended storage past this point may have decreased volatile concentrations. Therefore, sample hardness, melting point, polymorphic form, and length of time past transition to form VI may all be important characteristics in determining volatile release and flavor perception during sensory analysis of chocolate.

Although storage conditions caused several aldehydes and pyrazines known to affect chocolate flavor to significantly decrease or become undetectable, chocolate flavor as determined by the descriptive sensory panel was not significantly different between samples. Along with sample hardness and changes in crystal state associated with lipid polymorphism and fat bloom formation, this could also be due to these compounds not having a significant impact on chocolate flavor, inadequate panelist training, or a combination of SFC, melting point, and volatile release during mastication. The increased melting point associated with the polymorphic transition of chocolate stored at high temperature for 8 weeks without fluctuations or for 4 weeks with fluctuations may not have allowed complete melting during sensory analysis (i.e., body temperature in sensory versus 40 °C during instrumental analysis), thus affecting complete volatile release. Others have noted a discrepancy between in vivo and in vitro aroma analysis.[24] One issue between these two methods is that during instrumental volatile analysis, samples are allowed to melt completely and to reach equilibrium before results are attained, whereas in sensory panels, samples may not melt completely and equilibrium is not reached before an assessment is made. Gierczynski et al.[24] hypothesized that sensory panelists interact with the food in a much more dynamic way by adjusting their mastication pattern to the texture they are measuring. Similarly, Weel et al.[25] determined that sensory and instrumental results vary because of the psychosocial way that panelists use all senses to assess flavor perception when sample textures vary. The same study also determined that flavor perception by sensory panelists was affected more by the textural influence of mouthfeel, rather than by volatile concentrations alone.[25] Sensory analysis more closely resembles real-life results. Although there were significant changes in volatile concentrations during storage, chocolate flavor was not found to be significantly different by a highly trained descriptive sensory panel. Therefore, the average consumer also should not be able to detect a difference.

According to Roberts and Pollien,[26] the lipid phase in milk was the main influence on flavor volatility. Previously, SFC has been shown to influence flavor volatile loss, with a higher concentration of flavor compounds lost with higher SFC.[26,27] This may explain the volatile loss due to evaporation or oxidative reactions during low-temperature storage (−27.2 to 23.0 °C). Furthermore, the less time flavor compounds come in contact with the liquid portion of the lipid, the less volatiles are transferred from the aqueous to lipid phase, thus increasing volatile loss.[28] Maier[29] showed that liquid triglycerides bind significantly more aroma compounds than solid triglycerides. Cocoa butter and chocolate SFC vary slightly even during ambient storage.[30,31] Chocolate stored at room temperature (ambient and high relative humidity at 23.0 °C) and frozen conditions (−27.2 °C) would be expected to have a higher SFC during storage than samples stored at high temperature, thus facilitating volatile loss throughout storage at ambient, frozen, and high relative humidity conditions. This may explain why several pyrazines (2-ethyl-3-methylpyrazine and trimethylpyrazine) were not detected during storage in these conditions. Storage at high temperature with and without fluctuations would result in chocolate with a higher proportion of liquid and lower SFC than other storage conditions, increasing volatile transfer to the liquid lipid phase and thus significantly decreasing volatile release during storage. Cooling either by removal from a high storage temperature or through temperature fluctuations may entrap volatiles in the solid fat,[32] further decreasing volatile release throughout storage.

This is the first time a descriptive sensory panel and multiple instrumental analyses have been used to determine the impact of lipid polymorphism and fat bloom formation on volatile release in stored chocolate samples. The current study provides a good starting point for future studies to be conducted relating sensory and instrumental data to newly manufactured chocolate, which is currently underway in our laboratory. The results of this study are relevant to the chocolate industry, as large companies may do the research "in-house" but not publish the results and many small companies do not have the capacity to carry out research of their own.

Overall, chocolate stored at high temperature with and without fluctuations was the most visually and texturally compromised, but had the least amount of instrumental volatile change. Storage at ambient, frozen, and high relative humidity conditions caused an increase in volatile loss. Compositional rearrangements due to the polymorphic shift to form VI, sample hardness, and a lower SFC during storage may have increased volatile concentrations in instrumental analyses but decreased volatile release during sensory analysis. When all instrumental and sensory data had been taken into account, the storage conditions that had the least impact on texture, surface roughness, grain size, lipid polymorphism, fat bloom formation, volatile concentration, and sensory analysis were storage at constant temperature and 75% relative humidity.

AUTHOR INFORMATION

Corresponding Author

*Postal address: 208 Bevier, 905 S. Goodwin, Urbana, IL 61801. Phone: (217) 244-6788. Fax: (217) 265-0925. E-mail: engeseth@illinois.edu.

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Notes

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