Gas Chromatographic Analysis of Fragrances in Palm-Based White Soaps

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Four commercial soap fragrances 1, 2, 3, and 4 were incorporated in palm-based white soaps and made into bars. The soap bars were stored at 25°C and 40°C for three months. The headspace gas-chromatographic volatiles, fragrance odor intensity and whiteness analyses of the soap samples were carried out at 10-day intervals. By means of gas chromatography and mass spectrometry, some of the headspace gas-chromatographic peaks were identified. These peaks were useful in monitoring the fragrance components. For example, fragrance 4 contains the components limonene and citronellal which are known to possess a citrus odor component. Fragrance odor intensity scores showed that the intensity of the fragrances in the soap base was the same for all. Results also showed that most of the fragrances had the same effect on the whiteness of palm-based white soaps, except for fragrance 2 which gave a pink color to the white soaps. Equations were established between the fragrance odor intensity and area under the gas-chromatographic peaks. The equation can give useful information on the fragrance odor intensity by calculating the area of the chromatographic peaks; the fragrance odor intensity is a reflection of the fragrance retention in palm-based soaps.

KEY WORDS: Degree of whiteness, fragrance, headspace gas chromatography, odor intensity, palm-based soap.

The main function of fragrance is to mask the odor of the soap base. Consumers demand long-lasting fragrance in soaps, which means that the soap fragrance must be well retained in the soap base. However, addition of fragrance to soap can also give rise to discoloration as a result of the reaction between the alkaline soap base and the organic fragrance compounds. In some countries, consumers prefer high-quality white soaps as gifts, and therefore, the fragrance chosen for such soap should not affect its whiteness. Some fragrances in concentrated forms are highly colored and, therefore, can also affect the whitness of soap.

Gas chromatography has been extensively used in perfumery analysis. Grosso (1) discussed static headspace analyses of perfumery materials incorporated in finished products. Burrel (2) studied the behavior of perfumery ingredients in products by gas liquid chromatographic analysis. He developed a gas liquid chromatographic technique for analyzing perfumery ingredients directly from some products and found no interference from these bases. Correlation of the headspace gas-chromatographic profile and organoleptic score of various food systems has been widely studied and discussed (3–7). Parallel studies in scented soap are relatively limited in comparison. The aim of the present work was to investigate the effect of commercial fragrances on palm-based white soaps with storage time. An attempt was made to establish a relationship between the fragrance odor intensity and the area of volatile chromatographic peaks of the soap samples.

EXPERIMENTAL PROCEDURES

Preparation of perfumed soap samples. Commercial soap noodles (1040 g), obtained from Unichema Malaysia, (Selangor, Malaysia) were weighed out and the moisture content was measured. The soap noodles were ground into powder, and distilled water was added to obtain a moisture content of 12% (a moisture content of less than 12% will cause the soap to crumble). TiO_2 was added to 0.2%, followed by addition of 1.2% fragrance. The soap fragrances used were numbered 1,2,3 and 4. They were obtained from International Flavors and Fragrances (S) Pte. Ltd. (Singapore). The mixture was blended thoroughly so that the fragrance was uniformly distributed throughout the soap matrix. The mixture was then divided into equal portions of 80 g and compacted manually in a plastic wrapper. The compact balls (still wrapped in plastic wrapper) were molded and stamped to produce a soap bar. The bars were wrapped in glassine paper and boxed. Three sets of four bars each were kept at 0°C, 25°C and 40°C. Two bars were used for sensory evaluation and degree of whiteness test while the other two were used for headspace gas chromatographic volatiles analysis. The third set stored at 0°C was used as control. The evaluations were carried out at 10-day intervals over a period of three months.

Degree of whiteness. For evaluation of degree of whiteness of the soap samples, measurements were carried out with the Ogawa Seiki Nippon Denshoku color and color difference meter model 1001 DP (Tokyo, Japan), and the parameter was calculated based on the Hunter Whiteness equation:

$$W = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2} \qquad [1]$$

where W = degree of whiteness, L = reflection coefficient of samples at 457 nm (reflection coefficient of standard white sample at 457 mn is taken to be 100%), and a and b = color hues.

Headspace sampling. Collection of soap volatiles was carried out by means of a portable Hewlett-Packard Model 19395A headspace sampler (Palo Alto, CA) linked to a gas chromatograph (8,9). The headspace sampler had an automated valve and loop sampling system. A 1-mL sampling loop was installed in this model. The vial containing soap samples was thermostated in a carousel mounted on a silicon oil bath at 80°C and equilibrated for 30 min. The headspace sampling conditions were as follows: Valve and loop temperature 85°C, vial pressurization was 2.8 bar, carrier gas helium flow rate was 6 mL/min, pressurization time was 10 s, and venting time was 10 s. The soap volatiles collected in the sampling loop were injected into the gas chromatograph *via* the transfer line, which was heated at 85°C. The duration of injection was 3 min.

Gas chromatography and mass spectrometry. Analysis

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of the soap volatiles was carried out with a Hewlett-Packard gas chromatograph model 5890 (Palo Alto, CA). The gas chromatograph was equipped with a 60-m Supelcowax 10 capillary column (0.32 mm i.d. × 0.25 microns df film thickness) (Supelco, Bellefonte, PA). Column head pressure was 20 mL/min, split ratio 1:10 and flame-ionization detector make-up flow rate was 28 mL/min. Temperature programming was from 100°C to 180°C, increasing at a rate of 5°C/min and kept isothermally for 20 min. Detector and injector temperatures were 250°C.

For identification of volatile peaks, the headspace sampler was connected to a gas chromatograph-mass spectrometer. The system used is a JEOL Model JMS-D303 double-focussing mass spectrometer linked to a JEOL Data system model JMA DA-5000 (JEOL Ltd., Tokyo, Japan). The injector separator was at 250°C and the inlet ion source at 220°C. Ionication voltage was 70 eV and the carrier gas was helium at 40 mL/min.

Fragrance odor evaluation. Fragrance odor evaluation was conducted by ten panel members. The panel members were trained to recognize various odors such as citrus, herbal, floral, woody, mossy, musky, spicy following the method by Jellinek (10) on odor recognition. The odor intensity score of the fragrance in the soap sample was based on a hedonic scale of 1 to 9 for scoring of the strength of the fragrance. This was on the assumption that the pleasantness of the fragrance remained the same throughout storage time. The strength of the fragrance odor was scored as 0 for absent, 1 for slight, 5 for moderate and 9 for the strongest. A fragranced soap sample kept at 0°C was used as control and deemed representative of fresh sample. The fragranced soap samples were presented to the panel members at 10-day intervals. The panel members were instructed to use the control as a guide to judge the strength of the fragrance from the 10th day of storage until the end of the experiment.

Statistical analysis. The scores for the four fragrances with storage treatments under three temperatures were

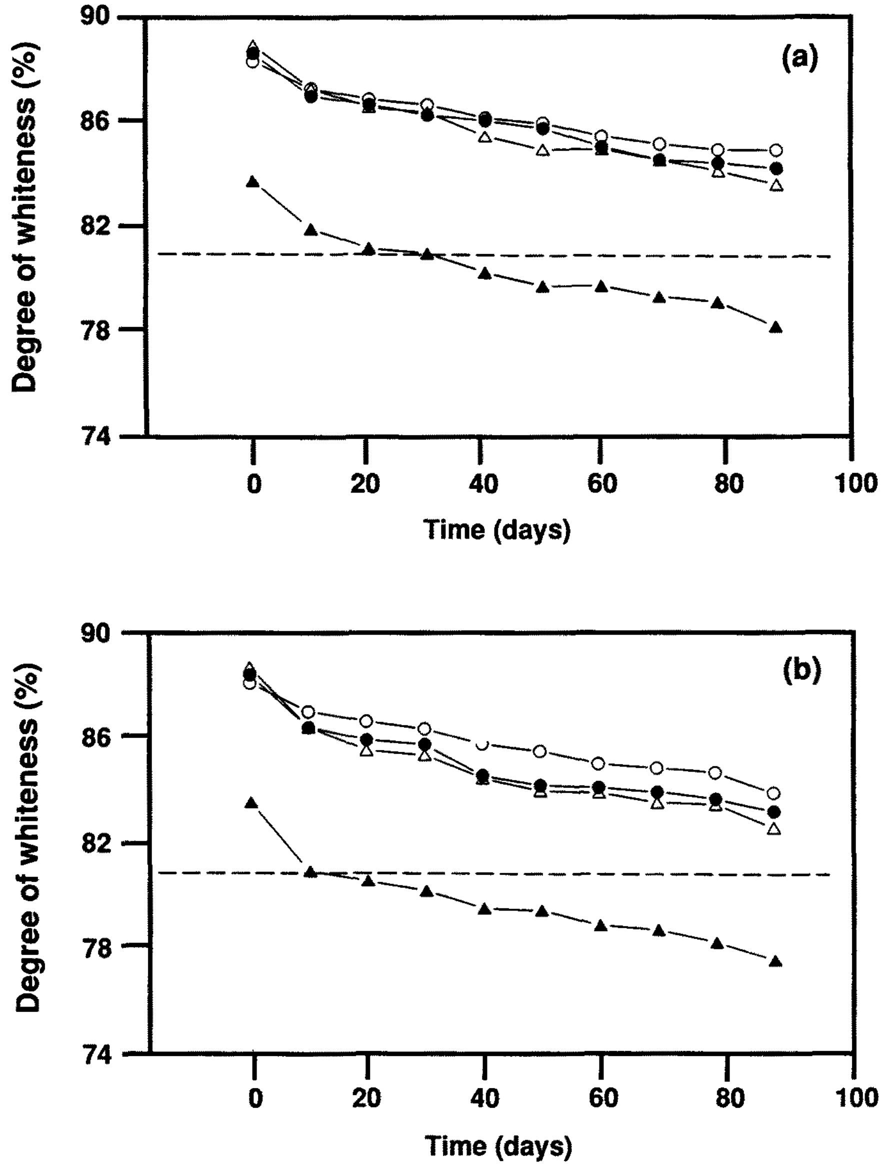

FIG. 1. Degree of whiteness of palm-based white soaps incorporated with four different fragrances stored at (a) 25°C and (b) 40°C for 90 days. Symbols: -O- = fragrance 1, -▲- = fragrance 2, -△- = fragrance 3, -●- = fragrance 4.

subjected to the analysis of variance (ANOVA). The relationship between the fragrance odor intensity scores and results of the gas-chromatographic instrumental analysis was established by regression analysis with a variable selection procedure.

RESULTS AND DISCUSSION

Degree of whiteness. Figure 1 shows the trend of degree of whiteness of fragranced soaps stored for 3 mon. In all soap samples, the degree of whiteness decreased with storage time and the decrease was slightly greater for soap samples stored at 40°C. Soap samples incorporated with fragrance 2 showed a marked reduction in the degree of whiteness. This is clearly shown in Figure 1 that at both temperatures of storage there are significant differences in the degree of whiteness throughout the storage period. In fact, the value decreased to 77.6% (80% is the minimum limit for a good pure white soap).

Headspace-gas chromatography-mass spectrometry. The headspace gas-chromatographic volatile profiles of the four fragrances incorporated in palm-based soaps are shown in Figures 2, 3, 4 and 5. Fragrances 1, 3 and 4 showed five major chromatographic peaks and fragrance 2 seven major peaks. These peaks are identified in Table 1.

Fragrance 1. Five peaks were clearly observed for this fragrance and marked as A, B, C, D and E. Peak A was difficult to identify while peak B was found to be *p*-mentha-8-en-3-ol. Peak C and D were monoterpene diols ($C_{10}H_{12}O_2$, $M^+ = 164$) and ($C_{10}C_{16}O_2$, $M^+ = 168$), respectively. The specific diol could not be identified positively due to lack of standard. E was identified as nerolidol. After 10 days of storage at 25°C, the amount of these compounds in the soap samples had decreased, as can be seen from the peak size on the 50th and the 90th day (Fig. 2) and a similar pattern was observed for the 40°C storage study. Study of the chromatographic profiles of soap stored at 25°C showed that peak B was the most suitable peak to be used to monitor the fragrance behavior because on the 90th day at 40°C storage the peak area was still 5333 units, while the other components were absent or zero.

Fragrance 2. Fragrance 2 was observed to have five prominent peaks, A, B, C, D and E. These peaks were *p*-mentha-1,8-diene (A); 2,5-dimethyl-2-hexanol (B); *p*-mentha-8-en-2-ol (C); 2-methyl-6-methylene-7-octen-2-ol (D); and benzyl acetone (E). Two other peaks, F and G, could not be identified but could also be important.

At 25°C storage temperature, the amount of each peak increased on the 10th day, then decreased progressively with storage time with the exception of peak D. In fact, based on peak area, the amount of peak D was inconsistent throughout storage time and would not be suitable as a monitoring tool. Storage at 40°C showed progressive decrease in the amount except peaks C and D. These increases in amount of C and D on the 20th day rendered them unsuitable for monitoring purposes at 40°C. In this case, peaks A, B, E and G were suitable to be used in monitoring the aging process of the soap.

Fragrance 3. The most important peak in the headspace of this fragrance was *p*-mentha-8-en-2-ol acetate (peak C). At 25°C storage temperature, the amount of peak C fluctuated at first but then decreased steadily with storage time. This could be due to nonuniform rate of release of

TABLE 1

Compounds from the Headspace of the Various Fragranced Soaps

Samples	Peaks	Retention time (min)	Compound
Fragrance 1	A	6.52	Unknown
	B	9.62	*p*-Mentha-8-en-3-ol
	C	12.56	Monoterpene diol ($C_{10}H_{12}O_2$, $M^+ = 164$)
	D	13.20	Monoterpene diol ($C_{10}H_{16}O_2$, $M^+ = 168$)
	E	16.92	Nerolidol
Fragrance 2	A	5.75	*p*-Mentha-1,8-diene
	B	6.64	2,5-Dimethyl-2-hexanol
	C	9.41	*p*-Mentha-8-en-3-ol
	D	12.59	2-Methyl-6-methylene-7-octen-2-ol
	E	13.93	Benzyl acetone
	F	13.93	Unknown
	G	17.02	Unknown
Fragrance 3	A	5.73	Terpene alcohol ($C_{10}H_{18}O$, $M^+ = 154$)
	B	9.36	Citronellal
	C	13.19	*p*-Mentha-8-en-2-ol acetate
	D	16.21	*trans*-Geraniol
	E	20.21	*p*-Anisaldehyde
Fragrance 4	A	5.77	Limonene
	B	6.62	2,5-Dimethyl-2-hexanol
	C	8.54	Citronellal
	D	12.22	2-Methyl-6-methylene-1,7-octadien-3-one
	E	13.16	Citral

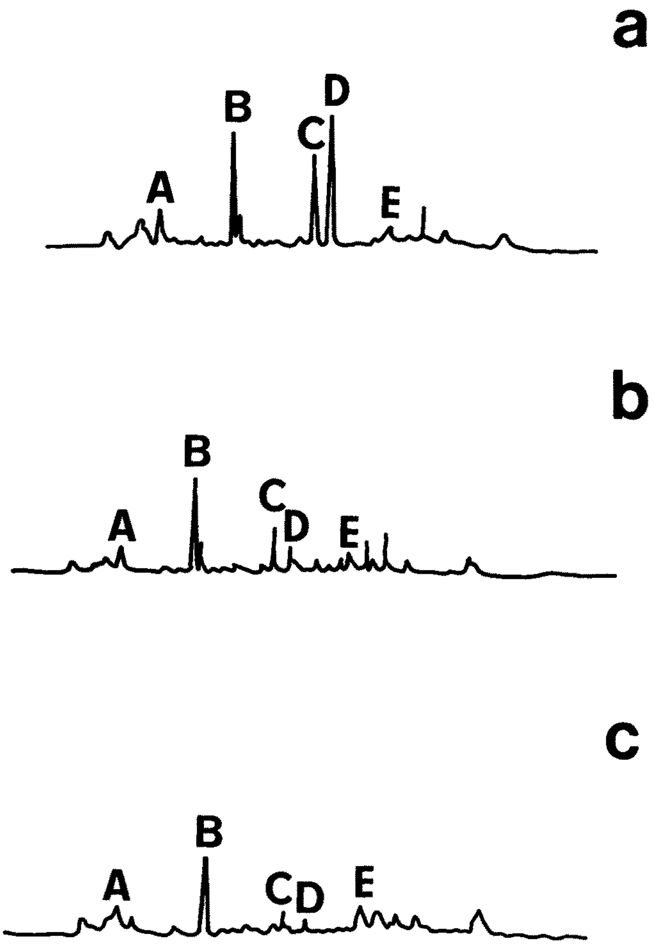

FIG. 2. Headspace chromatographic profiles of fragrance 1 in palm-based soaps. (a) 1st day, (b) 50th day and (c) 90th day of storage at 25°C. Symbols: A = unknown; B = *p*-mentha-8-en-3-ol; C = monoterpene diol ($C_{10}H_{12}O_2$, $M^+ = 164$); D = monoterpene diol ($C_{10}H_{16}O_2$, $M^+ = 168$) and E = nerolidol.

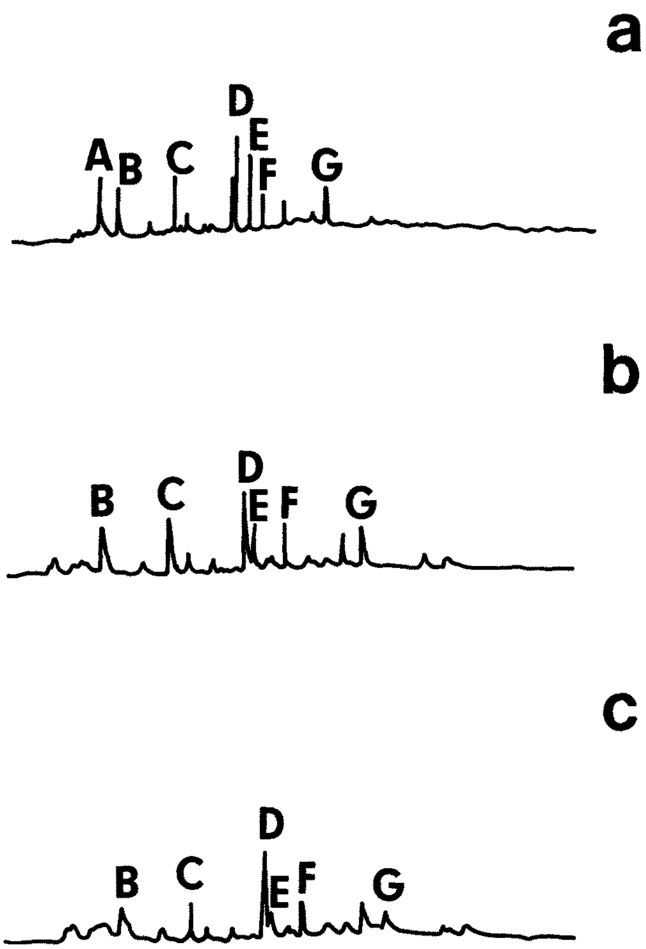

FIG. 3. Headspace chromatographic profiles of fragrance 2 in palm-based soaps. (a) 1st day, (b) 50th day and (c) 90th day of storage at 25°C. Symbols: A = *p*-mentha-1,8-diene; B = 2,5-dimethyl-2-hexanol; C = *p*-mentha-8-en-3-ol; D = 2-methyl-6-methylene-7-octen-2ol; E = benzyl acetone; F = unknown and G = unknown.

the perfume from the soap base. The same pattern was also seen with the other peaks D and E, which were *trans*-geraniol and *p*-anisaldehyde, respectively.

The most obvioius peak to be chosen for monitoring of the aging process would be peak C as it persisted until the 90th day of storage time.

Fragrance 4. The headspace analysis of this fragrance showed five peaks A, B, C, D and E, which were identified as limonene; 2,5-dimethyl-2-hexanol; citronellal; 2-methyl-6-methylene-1,7-octadien-3-one and citral, respectively.

During the first part of the storage period, the limonene peak (A) was dominant but declined in concentration with time while the citronellal peak (C) persisted in the soap and was dominant in the latter part of the study. Therefore, either limonene (peak A) or citronellal (peak C) can be used as a marker for the aging of this fragrance. This also indicates that for this fragrance the crossover from limonene odor to citronellal odor could take place from the 40th day onward. On the 90th day, the citronellal was still present and was most probably the main contributor of the fragrance odor.

Intensity score. Generally, the fragrance in soaps had a high initial fragrance odor intensity score (Fig. 6), especially fragrance 4 with a score of 7.5 (SD 1.513), and this implies that the top notes of fragrance 4 had a strong impact. The intensity decreased with time, reaching a minimum at 80 days of storage time, and this behavior was observed in all the soap samples except for the sample with fragrance 2 at 25°C storage and for soap sample with fragrance 1 at 40°C storage. After the 80th day, the intensity score increased and this reflects the typical behavior pattern of perfumery. The decrease and increase in intensity score with time indicate respectively the disappearance and appearance of the various notes, such as top note, middle note and base note. Soap samples incorporated with fragrance 4 illustrated this property well where the two peaks, limonene and citronellal, gave the subtle change in the citrus odor of the fragrance. Limonene could possibly be either the top note or middle note while citronellal could be the base note. The unexpected high scores in the fragrance odor intensity of fragrance 2 on the 80th day at 25°C and of fragrance 1 at 40°C

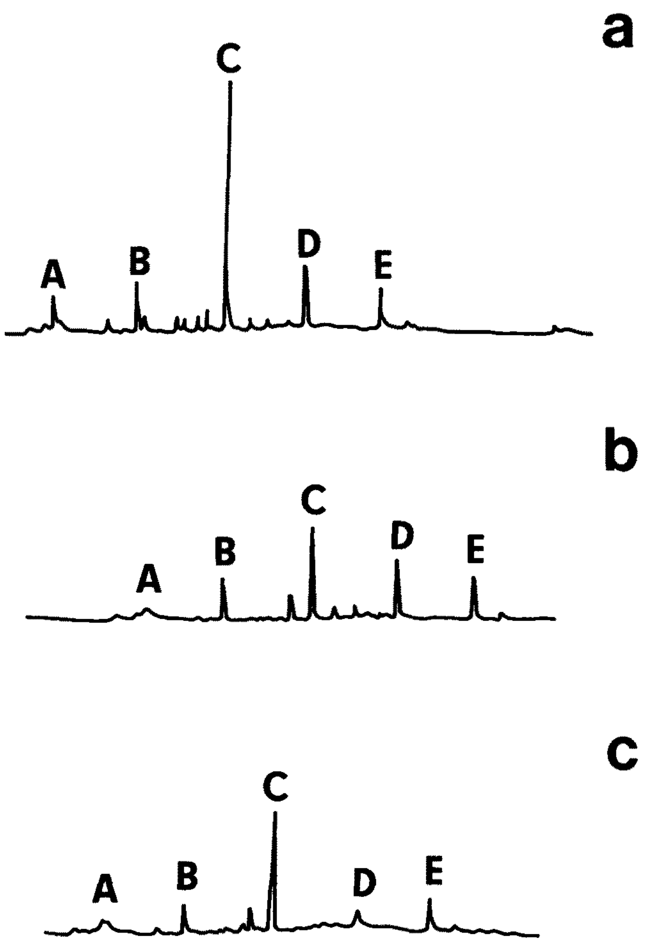

FIG. 4. Headspace chromatographic profiles of fragrance 3 in palm-based soaps. (a) 1st day, (b) 50th day and (c) 90th day of storage at 25°C. Symbols: A = terpene alcohol ($C_{10}H_{18}O$, $M^+ = 154$); B = citronellal; C = *p*-mentha-8-en-2-ol acetate; D = *trans*-graniol and E = *p*-anisaldehyde.

TABLE 2

Two-way Table of Means for Fragrance X Storage time (with SE of means and LSD)[a]

Storage time days (S)	Fragrance (F) 1	2	3	4	Mean
0	6.0000	5.5000	5.8000	7.5000	6.2000
10	6.6333	5.7000	5.7000	7.7333	6.4417
20	5.1000	4.8000	4.9667	6.3667	5.3083
30	5.2667	4.5333	4.6667	5.8000	5.0667
40	4.3333	4.2333	4.5667	5.2333	4.6667
50	4.9667	4.1333	4.3333	4.8667	4.5750
60	6.5000	6.0333	5.7333	6.3667	5.9083
70	5.3000	5.9333	6.2333	6.4333	5.9750
80	4.3333	6.7000	4.8000	7.0667	5.7250
90	4.9433	5.4633	4.9000	6.4667	5.4433
Mean	5.3677	5.2030	5.1700	6.3833	5.3100

[a] F means: SE = + 0.9440; LSD = 0.3706
F × S means: SE = + 0.2985; LSD = 0.8532
S means: SE = + 0.1493; LSD = 0.4703

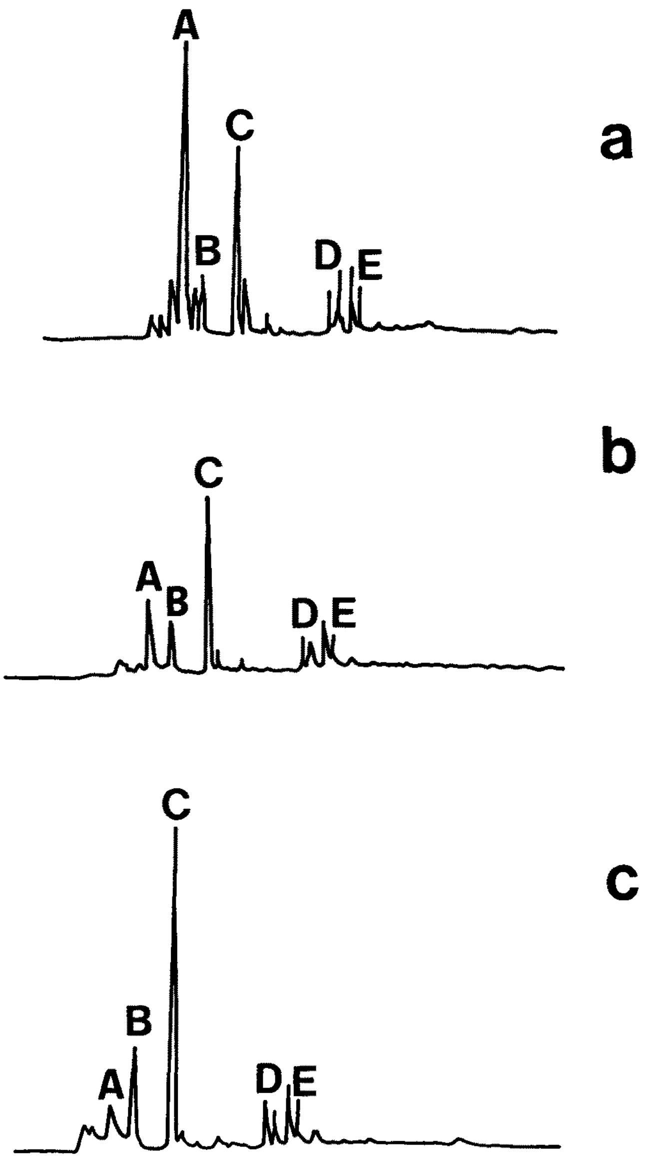

FIG. 5. Headspace chromatographic profiles of fragrance 4 in palm-based soaps. (a) 1st day, (b) 50th day and (c) 90th day of storage at 25°C. Symbols: A = limonene; B = 2,5 dimethyl-2-hexanol; C = citronellal; D = 2-methyl-6-methylene-1,7-octadien-3-one and E = citral.

TABLE 3

Table of Means

Temperature (T)	Mean	SE (LSD)
0°C	5.9590	+ 0.08176 (0.367)
25°C	5.5785	
40°C	5.0555	
Mean	5.5310	

could be due to a minor discrepancy in panel members' evaluation.

Analysis of variance and regression analysis. The results in Tables 2 and 3 show that the differences among

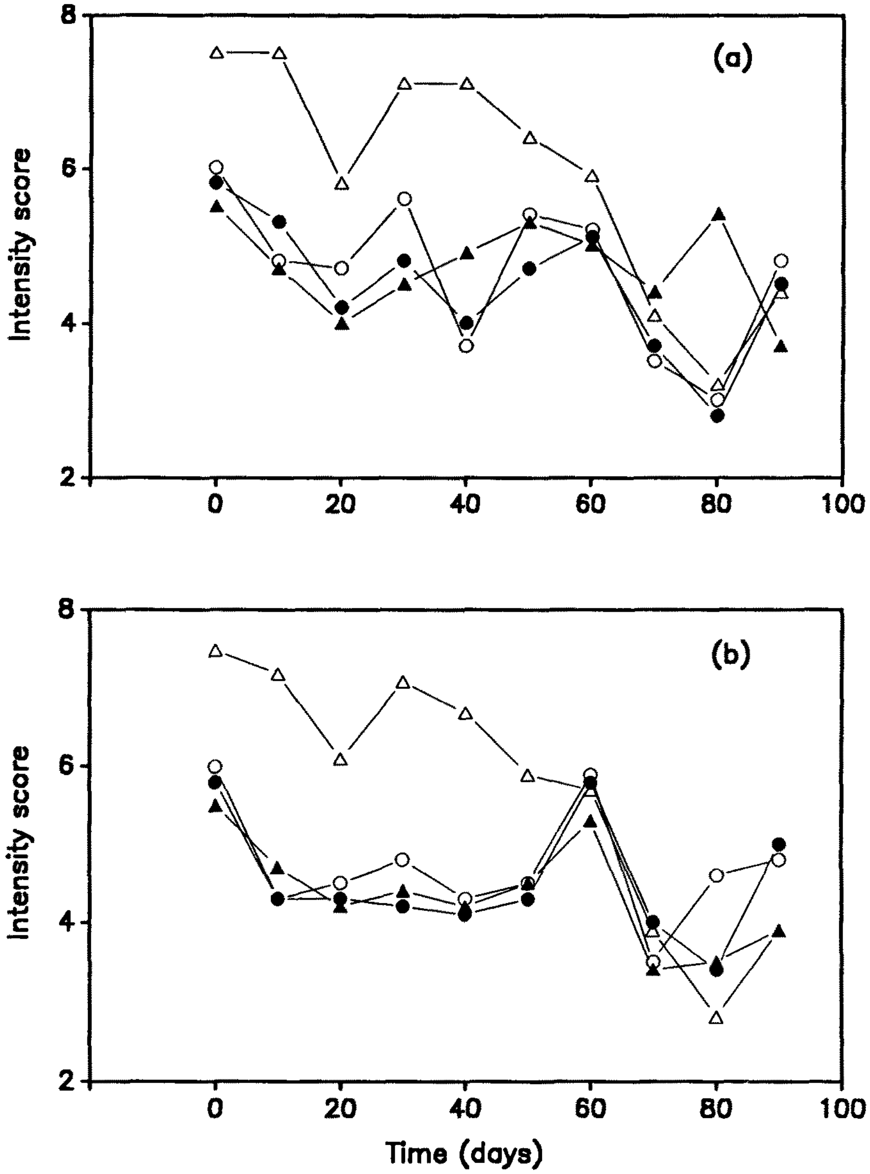

FIG. 6. Intensity score of four fragrances incorporated in palm-based soaps stored at (a) 25°C and (b) 40°C for 90 Days. Symbols: -○- = fragrance 1, -▲- = fragrance 2, -●- = fragrance 3 and -△- = fragrance 4.

the three factors of fragrance, temperature and storage were highly significant ($P < 0.01$). Significant interaction between fragrance and storage was also observed, implying that storage had different effects on the various fragrances incorporated in the soap. This difference may be due to fragrance 2, which also showed a marked difference in the decrease of degree of whiteness.

From the regression analysis, it was found that the relationship between fragrance odor intensity score (I) and headspace gas-chromatographic peak area (A–G) was likely to be nonlinear because interactions between chemical components appear to be substantial, as shown in Table 4. Table 5 shows the values of the actual fragrance odor intensity score and the calculated values. The difference between calculated and actual values was slight. Therefore, this relationship is useful in indicating the condition of the soap. The relationship is also useful because measurement of the gas-chromatographic peak area could indicate the intensity of the fragrance. The computed value of the fragrance odor intensity score can reflect the retention power of fragrance in soap because it was amount-dependent. A large chromatographic peak area means that a substantial amount or concentration of fragrance was still retained in the soap, hence high fragrance odor intensity score. A small chromatographic area means less fragrance was retained in the soap, which is reflected in a low fragrance odor intensity score.

Thus, in conclusion, fragrance and storage time had a significant effect on the degree of whiteness. The effect of storage time could be seen in the fragrance odor intensity score and was supported by the headspace gas-chromatographic profiles.

TABLE 4

Relationship Between Intensity Scores (I) and the Area of Chromatogrpahic Peaks A-G[a]

Fragrance 1:

I =	4.253 (2.89)[a]	+	3.432 E (1.21)	+	0.119 AD (−1.43)	−	0.0740 CD (−2.71)	−	0.611 BE (2.33)	+	0.292 BC (2.33)
+	0.467 AE (1.62)	−	0.365 AC (−2.27)	−	1.322 C (−2.37)	−	0.112 BD (−1.82)	+	0.441 DE (1.78)	+	0.0343 AB (1.05)

Error standard deviation = 0.834 (DF. 18)
R = 0.768[b]

Fragrance 2:

I =	2.892 (3.98)	+	0.248 A (−4.54)	−	2.454 A (5.54)	+	2.061 D (−5.89)	−	0.280 (4.73)	+	0.140 BG (4.73)
+	0.181 FG (3.58)	−	0.536 C (−1.48)	−	0.0365 AF (−3.82)	+	0.0517 CE (2.82)	+	0.121 DG (1.70)		

Error standard deviation = 0.519 (DF. 19)
R = 0.906

Fragrance 3:

I =	5.116	−	−0.00545 CE (−1.36)	+	0.131 E (1.33)	−	0.400 D (−3.3)	+	0.440 B (4.47)

Error standard deviation = 0.635 (DF. 25)
R = 0.712

Fragrance 4:

I =	5.219	+	0.157 A (3.76)	+	0.0243 AD (2.38)	+	0.0442 BC (−3.53)	+	0.061 CE (2.23)	+	0.516 BD (3.87)	+	0.136 BE (1.68)
−	2.898 D (−3.66)	−	0.920 DE (−3.26)	−	0.0237 AB (−3.10)	+	0.202 C (2.41)						

Error standard deviation = 0.682 (DF. 18)
R = 0.828

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[a] Numbers in parentheses represent the t value of the regression coefficient.

[b] R = multiple correlation coefficient.

TABLE 5

Observed and Estimated Fragrance Odor Intensity Values on the 1st, 50th and 90th Day of Storage at 25°C

Fragrance	Observed	Calculated
1st day		
1	6.0000	5.8817
2	5.5000	5.6259
3	5.8000	5.9315
4	7.5000	7.4477
50th day		
1	5.8000	4.5245
2	4.2000	4.6954
3	4.5000	5.1591
4	5.0000	5.0994
90th day		
1	4.9000	5.2332
2	5.4400	5.7204
3	5.2000	4.6973
4	6.5000	6.5493

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