TABLE III

Drying Properties of Tall Oil Fatty Acid-Betulinol Esters

	Treatment			
	25 C	100 C	150 C	Ultraviolet light
Dry to touch	2 hr	1.5 hr	0.5 hr	1 hr
Tack free	2 days	1.5 hr	0.5 hr	1 hr
Hard dry	2 days	1.5 hr	1 hr	1 hr

It was observed that betulin palmitate was compatible in this mixture, giving a free-flowing paste. The paste was homogenized in a three-roll mill and a 2-mm plastisol layer was cast into the mold and gelled for 10 mins at 180 C. The resulting film was soft, transparent and brown. After one month's storage at room temperature no exudation of betulin palmitate from the film was observed.

REFERENCES

1. Avela, E., and B. Holmbom, U.S. Patent 3,965,085.
2. Aslam, M., K. Alan and S. Akhtar, Pakistan J. Sci. Ind. Res. 2:31 (1965).
3. Erä, V., P. Jääskeläinen and K. Ukkonen, Angew. Makromol. Chem. 88:79 (1980).
4. Handbook of Chemistry and Physics, CRC Press Inc., Cleveland, 1977, p. C-208.
5. Erä, V., and K. Noronen, JAOCS 56:992 (1979).

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Correlation of Gas Liquid Chromatographic Volatiles with Flavor Intensity Scores of Stored Sunflower Oils

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ABSTRACT

Samples of sunflower salad oil from seed produced in the northern United States containing butylated hydroxyanisole, butylated hydroxytoluene, tert-butylhydroquinone and propyl gallate were stored in flintglass and amber bottles in the presence and absence of light for 16 wk. Using Dupuy's direct gas liquid chromatography method, correlations were made between pentane and flavor intensity scores and total volatiles and flavor intensity scores as well as flavor intensity values (FIV). Samples stored in clear bottles exposed to light had higher scores and pentane content than those stored in amber bottles or in the dark. Peroxide values were highest for samples stored in amber bottles. High correlation was found between flavor intensity scores and pentane and between pentane and FIV for rancid and painty flavor descriptors. Indications from the chemical and sensory data suggested that the addition of antioxidants had little effect on flavor scores when the oil was stored in the absence of light.

INTRODUCTION

Sunflower production in the United States has grown from somewhat less than a million acres in 1976 to over five million acres in 1979. With this expansion in production has come an increased and constant supply of oil for domestic use and the availability of sunflower salad oil on the grocer's shelves.

Initial oil quality, antioxidant efficacy and packaging affect flavor characteristics and ultimately consumer acceptance. One of the more recently developed methods for evaluating oil quality is the direct gas liquid chromatographic (GLC) measurement of volatiles (1,2). Several laboratories have used this approach, or variations thereof, as a quality control tool with a variety of products and have found high correlations with flavor scores (3-9).

In this study, a commercially prepared sunflower salad oil was evaluated by direct GLC, chemical analyses and by a sensory evaluation panel for the effects of antioxidants, packaging containers and light on oil quality. Correlations were established between volatiles and flavor intensity scores and flavor intensity values (FIV).

MATERIALS AND METHODS

Sample Preparation and Storage Treatment

The sunflower oil used was commercially prepared; contained no antioxidants; and had a fatty acid composition of 7.2% palmitic, 4.7% stearic, 14.0% oleic and 74.0% linoleic. Antioxidants were added to four 2.5-liter aliquots of oil at concentrations of 0.076% Tenox 6, 0.076% Tenox 26, 0.10% Tenox 20-A and a control containing no antioxidant. The Tenox series are mixtures of antioxidants (Eastman): Tenox 6 contains butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate; Tenox 26 contains BHA, BHT and tert-butylhydroquinone (TBHQ); Tenox 20-A contains only TBHQ. Flint clear glass and amber bottles (8 oz) washed with alcoholic KOH, dilute HCl and distilled water were filled with 180 ml of oil, their caps were tightened and the bottles were stored. For each antioxidant treatment, one set of four flint glass bottles was stored in the dark and a second set was stored on a rack exposed to light. In addition a third set of amber bottles were stored exposed to light. The light received was a combination of fluorescent and indirect sunlight with an intensity of 130 footcandles. Every 4 wk for 16 wk, samples stored in the dark and those stored in the light in clear and amber bottles for each treatment were frozen until chemical and sensory evaluations were conducted. Each oil sample was evaluated for Lovibond color, peroxide value (PV) (10), fatty acid composition (11) and direct GLC volatiles (12).

Gas Liquid Chromatography

Direct GLC volatiles were determined using a Tracor MT220 GLC equipped with a Hewlett Packard 3385-A integrator-recorder. A 6 ft. × 1/8 in. id stainless steel column packed with 7% Poly MPE on 60/80 mesh Tenax GC was used for the analysis. In preparing this column, chloroform, the solvent recommended by the manufacturer for Poly MPE, was found to polymerize the Tenax gas chromatography (GC) packing material. When ethylacetate

was used, no problems were encountered (5). Teflon "O" rings, septums, glass wool and glass sleeves were conditioned overnight at 220 C prior to use. Conditioned septums and "O" rings were used for only one run. Repeated usage resulted in volatiles being retained and appearing in subsequent runs. Septums and "O" rings could be re-used after conditioning.

Samples were prepared for GLC analysis by filling to the mark a 10-ml volumetric flask to which had been added exactly 1 ml of a volatile-free oil containing an internal standard, n-heptane, to give a final internal standard concentration in the sample of 0.5 ppm n-heptane. About 6 to 10 drops of this mixture were added to the top of the glass sleeve packed with glass wool, and the sleeve placed in the GLC inlet at 170 C. The sample was purged for 20 min with the column maintained at room temperature. The sleeve was removed and the oven was heated to a final temperature of 220 C at a rate of 5 C/min and held at 220 C until all peaks had eluted. Reproducibility was dependent on careful attention being paid to reproducing inlet temperature, purge time and cool-down rate before starting each run. Values for pentane and total volatiles were the average of 3 to 6 runs.

Sensory Evaluation

Ten panelists were selected for training. The training period lasted approximately 4 to 5 months with sessions held twice weekly. The basic purpose of the training was to teach sniffing and tasting techniques, detection and recognition of overall intensity, and detection and recognition of odor/flavor character notes. Samples used during training were sunflower oils, without antioxidants, stored at 60 C for 0, 2, 6, 8, 12 and 16 days. Other oils and various chemicals or materials were used occasionally to exemplify flavor descriptors (e.g., buttery, painty or rancid). Panel performance was evaluated and monitored throughout the study. Training was halted when $P \leq .05$ significance level for treatment differences for 3 replications was obtained.

Three separate groups of treated oils were evaluated. Identical sensory evaluation methods were used for each group. Samples within a group were presented to the panel three at a time. Oil samples were served at 50 to 55 C temperature in covered beakers (13). Panelists were instructed to remove the cover and sniff the odor of the samples from left to right and then rearrange them in the order of odor intensity from weakest to strongest. Panelists tasted the samples in this order (weakest to strongest odor), rinsing their mouths with warm water between tastes and allowing sufficient recovery time. Panelists evaluated the samples for intensity of the odor/taste sensation using a 1 (bland) to 5 (extreme), allowing for .5 unit increments. These values are defined as flavor intensity scores. In addition, panelists checked the appropriate flavor/odor descriptor (i.e., buttery, green beany, green creamy, nutty, rancid, or painty), and the intensity of the described character note, whether weak, moderate or strong. Flavor/odor description data were calculated as flavor intensity values (FIV) (14).

The first group of samples evaluated included sunflower oil with no antioxidants that had undergone accelerated storage at 60 C for 0, 2, 6, 8, 12 and 16 days. Each sample was evaluated 5 times by each panelist in an incomplete balanced design. Samples were presented three at a time, for 10 sessions, with each sample appearing with each other sample twice. Results from this group of samples showed the panel to have good discriminating ability and flavor intensity scores were used to establish a correlation with pentane and total volatiles in order to predict scores from GC volatile data in subsequent samples.

In the second group, samples in clear bottles with and without antioxidants and exposed to light for 4 and 16 wk were evaluated. These samples represent the extremes in light exposure and flavor deterioration which would be easily detected by the panel should actual differences exist. One of these 16-wk samples (Tenox 26, stored in the dark) was inadvertently used in this group. This was fortunate in that the data obtained from this sample allowed the use of a regression equation that better represented the relationship between flavor intensity scores and low pentane content. A third group of oils was evaluated to compare effects of storage in light and dark and the effects of bottle type. The oil containing no antioxidant was evaluated at 4 wk storage, Tenox 6 at 8 wk, Tenox 26 at 12 wk and Tenox 20-A at 16 wk. Each oil sample was evaluated by each panelist three times.

RESULTS AND DISCUSSION

Analysis of variance of intensity scores assigned to the accelerated storage samples by the individual panelists indicated discriminating ability of all panelists and significant differences among samples for intensity of odor/flavor impression. As storage time increased, the intensity of the odor/flavor impression of the sunflower oils increased. Increase in intensity corresponded to an increase in FIV for rancid and painty and a decrease in FIV for buttery and green beany.

Several studies have been conducted on oils, with and without antioxidants, using direct GLC analyses (2, 4, 9, 12). Our initial GLC analyses were conducted on those samples of sunflower oil which had undergone accelerated storage at 60 C. Pentane and other volatiles gradually increased with storage time with no interfering peaks. When commercial antioxidants were added, propylene glycol became a major limitation on individual peaks following propylene glycol. As its percentage in the oil increased, tailing became more severe and quantitation became more difficult. Therefore, correlations with flavor intensity scores were limited to pentane or total volatiles.

Pentane levels ranged from an initial value of 0.13 ppm to 3.01 ppm in the 16-day-old sample from the accelerated storage study. Total volatiles ranged from 0.76 to 6.95 ppm. A linear regression analysis, $y = mx + b$, gave an r^2 of 0.93 for pentane vs flavor intensity scores and 0.88 for total volatiles vs flavor intensity scores. Intensity scores of the oils assigned by the sensory panel increased as pentane and total volatiles increased. In this study, pleasantness or unpleasantness of the oil was not associated by the panel with odor/flavor intensity. High flavor intensity values in this study should not be confused with a scoring system where a high value is associated with a bland, pleasant-tasting oil.

Effects of antioxidant on oils exposed to light in clear bottles stored at room temperature to 4 and 16 wk are shown in Table I. At 4 wk there was no significant difference in flavor intensity scores for the four treatments; however, the Tenox 20-A sample had the highest score. At 16 wk, scores for all samples, except Tenox 26 (which was stored in the dark), were higher than at 4 wk but not significantly different. Because of the high correlation between pentane content and intensity scores for the accelerated stored oils, we calculated mean intensity scores by a linear regression equation. Predicted scores by the linear regression equation were .5 to 1 unit higher than actual scores. The best fit was obtained with a regression equation, $y = a + b(\ln x)$, using the log of pentane content (x) vs intensity scores (y) for samples exposed to light.

The effects of bottle type and storage in light or dark

TABLE I

Pentane and Flavor Intensity Scores of Samples Stored for 4 and 16 Weeks in Clear Bottles, Exposed to Light

Samples	Storage time (wk)	Pentane, ppm	Flavor intensity scores	
			Actual	Calculated[a]
No antioxidant	0	0.13	2.17	1.50
	4	0.61	2.92	2.97
	16	1.18	3.63	3.60
Tenox 6	0	–	–	–
	4	0.58	2.85	2.92
	16	0.80	3.32	3.23
Tenox 26	0	–	–	–
	4	0.43	2.93	2.63
	16[b]	0.25	1.90	2.09
Tenox 20-A	0	–	–	–
	4	1.28	3.70	3.68
	16	1.62	3.72	3.91

[a]Scores calculated using the formula y = a + b(1n x) (y = intensity score, x = pentane content).

[b]Samples stored in the dark for 16 weeks.

were evaluated with the third group of oils. To complete the data set, scores from the oils stored in light in clear bottles 4 wk and 16 wk were included for analysis using a general linear model (GLM). Table II shows pentane content, actual panel scores and predicted scores calculated from the regression equation for light-exposed samples. There were significant differences among oils due to bottle type and light conditions ($P < 0.01$). Predicted scores were generally within .5 units of actual panel scores. Samples stored in clear bottles in light received higher intensity scores than samples stored in amber bottles exposed to light or clear bottles in the dark. Within each bottle/light set, storage time significantly affected only those samples stored in clear bottles exposed to light.

FIV (not shown) were similar to those samples which had undergone accelerated storage. As pentane content and flavor intensity values increased, FIV for buttery decreased and FIV for rancid and painty increased.

Correlation coefficients were calculated for flavor intensity scores, FIV and pentane contents (Table III).

TABLE II

Pentane and Mean Flavor Intensity Scores of Stored Sunflower Oils

Antioxidants	Storage time (wk)	Storage conditions								
		Dark			Light					
					Bottle type					
					Clear			Amber		
		Pentane, ppm	Actual score[a]	Calculated[b] score	Pentane, ppm	Actual score[a]	Calculated score	Pentane, ppm	Actual score[a]	Calculated score
None	4	0.56	2.26[ab]	2.88	0.61	2.92[a]	2.97	0.45	1.83[b]	2.67
Tenox 6	8	0.23	1.63[b]	2.03	0.96	3.96[a]	3.40	0.42	2.42[b]	2.61
Tenox 26	12	0.26	2.18[b]	2.14	0.91	4.22[a]	3.35	0.59	2.37[b]	2.93
Tenox 20-A	16	0.58	2.44[a]	2.92	1.62	3.72[a]	3.91	0.48	2.46[a]	2.73

[a]Mean scores in rows having different superscripts are significantly different ($P = 0.05$).

[b]Scores calculated using the formula y = a + b(1n x) (y = intensity scores, x = pentane content) based on samples stored for 4 and 16 weeks (Table I).

TABLE III

Correlation Coefficients of Intensity Scores, Flavor Intensity Values and Pentane Content for Stored Sunflower Oils[a]

	Intensity score	FIV					
		Buttery	Green, beany	Green, creamy	Nutty	Rancid	Painty
FIV							
Buttery	-88						
Green, beany	-.01	-.04					
Green, creamy	.20	-.01	-.17				
Nutty	-.70	.59	-.13	-.08			
Rancid	.94	-.84	-.12	.11	-.76		
Painty	.95	-.89	-.04	.16	-.69	.86	
Pentane	.65	-.57	-.06	-.01	-.56	.74	.51

[a]Correlation coefficients exceeding 0.50 are significant at or below the 1% level.

A correlation had to exceed r=0.50 to be significant at the 1% level. FIV for green beany and green creamy were not significantly correlated with any other parameters. Possibly, these terms are not meaningful or appropriate in describing flavor change in sunflower oils, or the panelists could not detect these character notes, if present. Panelists had difficulty grasping the meaning and use of these terms when applied to sunflower oil during training and may have avoided using them in actual testing. Negative correlations were obtained for buttery and nutty FIV vs flavor intensity scores. Fresh oils have low odor/flavor intensities and the odor/flavor note is described as buttery. As the intensity of the overall odor/flavor impressions increased, FIV for rancid and painty also increased. Pentane was more highly correlated with rancid FIV (r=0.74) than with any other FIV response or flavor intensity score (15).

Antioxidant type and light had no effect on fatty acid composition during 16 wk of storage. Samples stored in clear bottles and exposed to light were visibly lighter than initial samples and generally showed loss of Lovibond red, while those stored in the dark showed an increase in yellow (Table IV).

Generally, samples containing Tenox 6 had the highest PV (Table V). Samples with Tenox 26 and Tenox 20-A had about equivalent values and were in general no higher than the control sample with no antioxidant. Both antioxidants contain TBHQ and appear to be better than BHA, BHT and propyl gallate in preventing peroxide formation. Samples stored in amber bottles had about equal PV regardless of antioxidant type. In addition, these values were higher than those for the light-exposed samples, probably due to the amber glass protecting the peroxides from further photochemical degradation (16).

Table V shows the amount of pentane found in the stored samples. As expected, the pentane content of those samples stored in the dark was generally lower than for those exposed to light and the addition of antioxidants was not beneficial in retarding pentane formation. Samples exposed to light in clear bottles contained the highest levels of pentane. Pentane content also generally increased with storage time. It is difficult to evaluate antioxidant efficacy since antioxidants are not generally effective in preventing the decomposition of the peroxide once formed (17). However, higher pentane content would possibly be related to high initial peroxide values. The higher pentane content in samples stored in clear bottles exposed to light than in samples stored in the dark or in amber bottles in the light would be a result of photochemical decomposition of the peroxides (7,17-19).

Generally, samples stored in the dark or protected from light by amber bottles had statistically insignificant differences in flavor scores which were not statistically different from fresh oil (intensity score = 2.17). In addition, antioxidants did not appear to show significant flavor protec-

TABLE IV

Lovibond Color of Sunflower Oils Stored in Clear Bottles

Storage conditions	Treatments			
	No antioxidant	Tenox 6	Tenox 26	Tenox 20-A
Initial	1.OY 0.2R	1.OY 0.2R	1.OY 0.1R	1.OY 0.2R
16 wk/dark	2.OY 0.2R	2.OY 0.2R	2.OY 0.3R	2.OY 0.1R
16 wk/light	1.OY 0.0R	1.OY 0.0R	1.OY 0.1R	1.OY 0.0R

TABLE V

Peroxide Values and Pentane Levels (ppm) of Stored Sunflower Oils

	Storage in dark		Storage in light			
			Clear glass		Amber glass	
Antioxidant	Peroxide values	Pentane	Peroxide values	Pentane	Peroxide values	Pentane
None						
0 wk	1.1	0.13	1.1	0.13	1.1	0.13
4	1.7	0.56	0.3	0.61	2.0	0.45
8	2.1	0.20	8.4	0.73	3.4	0.23
12	2.6	0.30	7.7	1.01	7.8	0.61
16	4.1	0.30	8.3	1.18	9.8	1.00
Tenox 6						
0 wk	1.0	0.13	1.0	0.13	1.0	0.13
4	8.7	0.24	14.3	0.58	2.0	0.18
8	2.2	0.23	9.8	0.98	4.0	0.42
12	3.9	0.28	9.3	1.19	11.4	0.51
16	6.1	0.61	2.3	0.80	11.4	0.69
Tenox 26						
0 wk	0.9	0.13	0.9	0.13	0.9	0.13
4	1.2	0.38	1.5	0.43	11.9	0.41
8	1.6	0.49	2.5	0.68	9.2	0.24
12	2.3	0.26	4.7	0.91	8.7	0.59
16	3.5	0.25	5.4	0.92	9.2	0.51
Tenox 20-A						
0 wk	1.0	0.13	1.0	0.13	1.0	0.13
4	1.4	0.51	1.8	1.28	9.8	0.67
8	1.6	0.78	2.0	1.24	12.2	0.58
12	2.1	0.43	3.4	1.20	8.7	0.37
16	2.2	0.58	4.1	1.62	9.1	0.48

tion. Those samples stored in clear bottles did, however, exhibit a significant rise in flavor intensity scores which did not appear to be altered by the addition of antioxidants.

REFERENCES

1. Dupuy, H.P., S.P. Fore and L.A. Goldblatt, JAOCS 48:876 (1971).
2. Dupuy, H.P., S.P. Fore and L.A. Goldblatt, Ibid. 50:340 (1973).
3. Legendre, M.G., H.P. Dupuy, R.L. Ory and W.O. McIlrath, J. Agric. Food Chem. 26:1035 (1978).
4. Dupuy, H.P., E.T. Rayner and J.I. Wadsworth, JAOCS 53:628 (1976).
5. Fore, S.P., M.G. Legendre and G.S. Fisher, Ibid. 55:482 (1978).
6. Rayner, E.T., J.I. Wadsworth, M.G. Legendre and H.P. Dupuy, Ibid. 55:454 (1978).
7. Warner, K., C.D. Evans, G.R. List, H.P. Dupuy, J.I. Wadsworth and G.E. Goheen, Ibid. 55:252 (1978).
8. Jackson, H.W., and D.J. Giacherio, Ibid. 54:458 (1977).
9. Waltking, A.E., and H. Zmachiniski, Ibid. 54:454 (1977).
10. Official and Tentative Methods of the American Oil Chemists' Society, Vol. 1, Third Edition, AOCS, Champaign, Illinois, 1964 (revised to 1978) Methods Cc 136-45 and Cd 8-53.
11. Metcalfe, L.D., A.A. Schmitz and J.R. Pelka. Anal. Chem. 38:514 (1966).
12. Dupuy, H.P., E.T. Rayner, J.I. Wadsworth and M.G. Legendre, JAOCS 54:445 (1977).
13. Moser, H.A., H.J. Dutton, C.D. Evans and J.C. Cowan, Food Tech. 4(3):105 (1950).
14. Cowan, J.C., C.D. Evans, H.A. Moser, G.R. List, S. Koritala, K.J. Moulton and H.J. Dutton, JAOCS 47:470 (1970).
15. Warner, K., C.D. Evans, G.R. List, B.K. Boundy and W.F. Kwolek, J.Food Sci. 39:761 (1974).
16. Lundberg, W.O., in "Symposium on Foods: Lipids and Their Oxidation," edited by H.W. Schultz, E.A. Day and R.O. Sinnhuber, A01, Westport, CT, 1962, p. 31-50.
17. Labuza, T.P., CRC Critical Review in FoodTechnology, Oct. 1971, p. 374,380.
18. Sattar, Abdus, and J.M. Denman, JAOCS 54:473 (1976).
19. Williams, J.L., and T.H. Applewhite, JAOCS 54:461 (1977).

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Distribution of Aflatoxin-Containing Cottonseed within Intact Locks

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ABSTRACT

Location of aflatoxin-containing seeds within locks of *Aspergillus flavus* contaminated bolls was determined. Of the 141 seeds examined from 22 intact locks, 78 exhibited bright greenish yellow fluorescence (BGYF) on the linters. Twenty-four seeds contained aflatoxins ranging from 0.231 to 151.3 μg of toxin per gram of seed. Twenty-one of these aflatoxin-positive seeds had linters exhibiting BGYF, and three had nonfluorescent linters. With one exception, aflatoxin contamination was concentrated in only one or two highly contaminated seeds in the apex half of tight locks, and the rest of the three to five seeds were negative. Explanations for this type of infection are discussed.

INTRODUCTION

Aflatoxin contamination in freshly harvested cotton is associated with lint that is bright greenish yellow fluorescent (BGYF) when viewed in long wave ultraviolet (UV) (1). In 1977 Lee et al. reported on the elimination of aflatoxin contamination by removal of all BGYF locks before ginning (2). In 1978 Lee and Cucullu reported on the aflatoxin content of individual cottonseed separated from BGYF locks by ginning (3). They found the highest concentration of aflatoxin in five seeds with BGYF linters. No attempt was made in that study to determine whether the five seeds were from the same lock or whether one or two seeds from several locks were contaminated.

The present study was undertaken to determine both the distribution and location of aflatoxin contaminated seeds within a lock. Such information will aid in establishing the mode of infection of cottonseed by toxin-causing fungi in the field.

EXPERIMENTAL PROCEDURES

Approximately 1 kg of Hopeville cotton was hand-harvested at the University of Arizona Experiment Station at Yuma, AZ, in the late fall of 1978, a year of high aflatoxin contamination (4). The field had a high incidence of pink boll worm infection; humidity under the canopy was also high. Peduncles and carpels were harvested with the intact bolls. Bolls and separated locks were examined in long wave UV and inspected for insect damage. Bolls containing any locks with BGYF, separated BGYF locks, or locks with lint that appeared brown-organge in UV were separated from the bulk of the sample. When possible, locks were identified with particular bolls. Seeds from locks separated for study were hand-ginned and their position in each lock recorded. Presence or absence of BGYF linters (fuzz) was noted; whole fuzzy seeds were then weighed and analyzed individually for aflatoxins (5). Separated lint adjacent to several aflatoxin-positive seeds was also analyzed by a slight modification of the same procedure. In this modification the lead acetate precipitation was eliminated. Lint from one seed that contained all four toxins was cultured on Blakeslee's Malt Agar (BMA). Dry spores of the cultured mold were inoculated onto autoclaved cottonseeds and seeds were incubated for seven days at 27 C and assayed for aflatoxins (6).

RESULTS AND DISCUSSION

The 22 locks separated because of fluorescence of any part of the bolls in UV (Table I) comprised 3% of the sample. Nineteen of the 22 locks were tight; three were fluffy locks (E-2, E-3, E-4) from a boll with one tight BGYF lock (E-1). This tightness could have been caused by mold infection (7,8). Only 12 locks could be identified with four bolls (C, D, E, and M). Because there were no fluffy fibers to cling to carpel walls, tight locks were easily separated and under normal field conditions could have fallen to the