dicated no dependence on protein concentration (Figure 7). Turbidity of conalbumin occurred at about pH 9 by heating (Figure 6), but the result of RCAM-egg albumin showed no increase of absorbance at about pH 9. It seems probable that soluble ovalbumin under around pH 9 dissolves conalbumin by functioning like a salt. Coagulation of CNBr-egg albumin was completely independent of protein concentration (Figure 7). As mentioned above, concentration-dependent coagulation of egg albumin may occur due to the intermolecular interactions between ovalbumin and conalbumin. Coagulation of proteins with low molecular weights indicated no dependence on protein concentration even when having a high content of hydrophobic groups (above 34%) (Figure 8). Consequently, concentration dependence seems to require two aspects of coagulable conditions. The protein should have a high molecular weight above 60 000 and a high content of hydrophobic groups.

From the results obtained here, it is possible to predict that wheat protein, one of the typical food proteins, has an excellent thermocoagulable property with concentration dependence because the mole percent of hydrophobic groups calculated from the amino acid composition (Tkachuk and Irvine, 1969) is 32.5%. Actually, wheat protein is largely utilized as a food ingredient due to its excellent thermocoagulable property.

If the amino acid composition of an unknown protein is determined, the thermocoagulable properties of the protein can then be predicted.

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Effects of Storage Temperature and Duration on Total Vitamin C Content of Canned Single-Strength Grapefruit Juice

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Commercially canned single-strength grapefruit juices were stored at 10, 20, 30, 40, and 50 °C. Their "total active" vitamin C (TAVC) contents [L-ascorbic acid (AA) plus dehydroascorbic acid (DHA)] and diketogulonic acid (DKA) contents were evaluated at 3-week intervals. At the end of 12 weeks the loss of TAVC ranged from less than 3% at 10 °C to greater than 68% at 50 °C. AA was continuously lost during the storage time, and the rate of loss increased as storage temperature increased. Polynomial expressions were calculated for the degradation rate of AA at each storage temperature. Large levels of DHA and DKA were not observed in stored canned juices because breakdown of AA apparently proceeded by an anaerobic pathway. The levels of DHA and DKA remained essentially unchanged during the 12-week storage period.

Grapefruit juice is a refreshing beverage with nutritional benefits as well-known as those of orange juice. One of the major nutritional values of grapefruit juice is its vitamin C content. Vitamin C potency (antiscorbutic activity) of natural products is based on the combined levels of L-ascorbic acid (reduced form of vitamin C, or AA) and dehydro-L-ascorbic acid (oxidized form of vitamin C, or DHA). Therefore, the combined contents of AA and DHA in a food product can be considered as "total active vitamin C" (TAVC). Under aerobic conditions, DHA is the first breakdown product of AA (Bauernfeind and Pinkert, 1970). DHA potency is considered between 75% and 100% of AA potency (Mills et al., 1949).

The official AOAC procedure (AOAC, 1970) for vitamin C analysis involves an indophenol dye titration procedure, which measures only the reduced form of vitamin C. For TAVC to be measured a procedure using 2,4-dinitrophenylhydrazine (2,4-DNPH) must be used in addition to indophenol titration. 2,3-Diketo-L-gulonic acid (DKA), a

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Table I. Some Chemical and Nutritional Properties of Processed Single-Strength Grapefruit Juice Prior to Storage

plant	pH	% citric acid	°brix	°brix/acid	ascorbic acid, mg %	dehydro-ascorbic acid, mg %	diketo-gulonic acid, mg %	total active vitamin C, mg %
A	3.60	0.89	9.23	10.37	33.59	0.81	0.57	34.40
B	3.60	0.90	9.66	10.73	35.77	1.23	0.47	37.00
C	3.54	1.23	11.72	9.53	35.00	0.89	0.54	35.89
D	3.56	0.94	9.66	10.28	35.38	1.21	0.45	36.59
E	3.55	0.94	10.06	10.70	35.38	0.72	0.68	36.10
av	3.57	0.98	10.07	10.29	35.02	0.97	0.56	36.00

further breakdown product of AA, is also measured by this TAVC method. Although DKA has no vitamin C activity its level of occurrence is often reported as a guide to vitamin C breakdown.

Loss of AA in single-strength grapefruit juice (SSGJ) has been shown to be related to storage time and temperature (Moore et al., 1944; Riester et al., 1945; Lamb, 1946; Moschette et al., 1947; Sheft et al., 1949; Moore, 1949; Freed et al., 1949; Heikal et al., 1967). In those studies, however, TAVC was not determined and most of those studies did not cover a wide range of storage temperatures. Temperatures should not only include those of normal storage (15–25 °C) but also those attained during adverse conditions. In a nationwide survey, several researchers (Moschette et al., 1947, Freed et al., 1949) reported that the temperature of non-air-conditioned warehouses ranged from below freezing to over 35 °C. Recent information (Ratcliff, 1978) indicates that warehouse temperatures in Florida range as high as 65 °C. A comparative study of TAVC at a wide range of feasible storage temperatures has apparently never been undertaken. Such a study should benefit both the consumer and the processor. The present study was conducted on commercially canned SSGJ prepared during the 1974 processing season to determine the effects of storage temperature and duration on retention of TAVC potency.

EXPERIMENTAL SECTION

Juice Samples and Chemicals. Commercially processed, canned single-strength grapefruit juice was obtained in May from five processors identified as A, B, C, D, and E. The capacity of the cans from A, B, C, and E was 1.36 L (46-oz) and that of the cans from D were 0.18 L (6-oz). All were tin-plated steel cans which were unlacquered (except on the ends). The cans were taken directly from the production lines and placed in a laboratory cold locker at −18 °C until the storage tests were initiated. The chemicals used for analysis were all certified ACS reagents with the exception of H_2S gas which was technical grade.

Storage of Juice Samples. Four cans of SSGJ from each of the five different processing plants were stored at 10, 20, 30, 40, and 50 °C (total of 100 cans). One can from each plant, at each temperature, was removed every 3 weeks. After removal, the cans were placed in a chilled locker at 2 °C. Analysis of the samples was completed as quickly as possible after removal from storage.

Analysis of Juice Samples. Degrees Brix (Soluble solids), percent citric acid, pH, and the degrees brix–acid ratio (degrees brix/acid) were determined by official analytical methods (AOAC, 1970) on juice from each source prior to storage. Percent citric acid and pH were also measured on samples of SSGJ stored at 50 °C after 12 weeks. A combination of the indophenol titration method (AOAC, 1970) and the 2,4-dinitrophenylhydrazine method (Roe, 1954; Roe et al., 1948) was used to determine vitamin C retention.

Three determinations (run in duplicate) were conducted on each SSGJ sample. The first determination, AA analysis, involved titration of the juice in a metaphosphoric–acetic acid (HPO_4–HOAc) buffer with a 2,6-dichlorophenol indophenol dye solution (AOAC method). The potentiometric endpoint determinations of Spaeth et al. (1962) was used in all the analyses because it can be detected more precisely than the normal visual color change associated with the endpoint, and commonly used with the AOAC method, especially in colored juices. The second determination analyzed for combined dehydroascorbic and diketogulonic acids. The juice was mixed with HPO_4–HOAC buffer, then with 2,4-DNPH, and heated (37 °C) for 6 h. After the addition of 85% H_2SO_4, the hydrazone content of the juice was determined colorimetrically. The third determination, DKA analysis, was performed by bubbling H_2S through the sample to reduce DHA to AA. After the reduction, DKA was determined by the 2,4-DNPH method (second determination). In effect, AA was analyzed by the first determination, DHA by the second determination minus the third determination, and DKA by the third determination. After AA, DHA, and DKA were evaluated, TAVC could be calculated as follows: TAVC = AA + DHA = detn. 1 + detn. 2 − detn. 3. AA, DHA, DKA, and TAVC were expressed as mg/100 mL of juice (mg %). Stannous chloride and thiourea were used in all 2,4-DNPH analyses to prevent oxidations of AA to DHA during the analysis.

Analysis of Results. The Q_{10} (the increase in rate for a 10 °C increase in temperature) and the E_a (activation energy) were calculated from the data. Rate equations for breakdown of AA were calculated by linear, logarithmic, and orthogonal polynomial regressions analysis (Hicks, 1973).

RESULTS AND DISCUSSION

Table I shows that the SSGJ samples from the five processors were similar. The values for pH and citric acid content of the SSGJ samples stored at 50 °C for 12 weeks showed no change in pH or citric acid percentage. Therefore, apparently, the acid environment of the juices did not change during storage.

The rate and mode of vitamin C breakdown in juice products is dependent on many factors: pH, presence of catalysts (trace metals), storage temperature, concentration of vitamin C, type of system (oxidative–reductive), and the presence of oxygen (Bauernfeind and Pinkert, 1970). In this study, storage time and temperature were varied so that the change in vitamin C retention could be observed. The other factors remained constant for all samples and did not affect the results. The most common pathway cited in the literature for vitamin C breakdown under aerobic conditions and an acid environment (pH 4) is mild oxidation. By this pathway, AA is oxidized to DHA; and this is followed by ring cleavage and the addition of H_2O to form DKA. Under anaerobic conditions, that pathway is not favored; instead, AA degrades by several steps to form furfural (Bauernfeind and Pinkert, 1970). Finholt et al. (1965) showed that in a hydrogen-ion-catalyzed anaerobic environment, furfural formed at a rate equal to that at which AA disappeared.

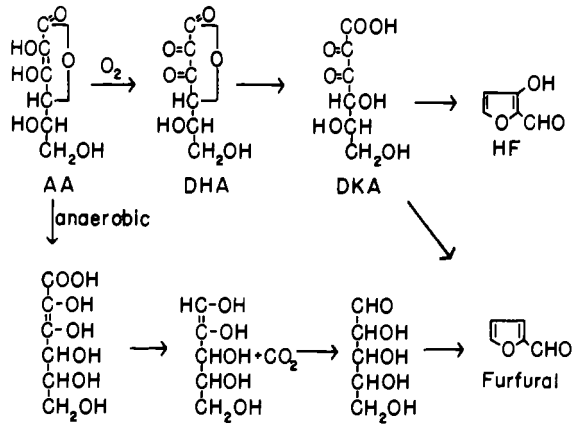

Figure 1. Ascorbic acid degradation pathways: AA, ascorbic acid; DHA, dehydroascorbic acid; DKA, diketogulonic acid; HF, hydroxyfurfural.

Table II. Total Active Vitamin C Retention and Ascorbic Acid (AA) and Dehydroascorbic Acid (DHA) Percentage of the Activity

time, weeks	temp, °C	% reten	% of TAVC	
			AA	DHA
0		100	97.3	2.7
3	10	98.6	98.4	1.6
	20	98.0	98.3	1.7
	30	97.9	97.4	2.6
	40	92.8	97.4	2.6
	50	78.0	97.3	2.7
6	10	99.0	97.5	2.5
	20	97.9	97.0	3.0
	30	96.1	97.7	2.3
	40	97.1	97.1	2.9
	50	59.3	95.3	4.7
9	10			
	20	96.6	98.3	1.7
	30	92.9	96.9	3.1
	40	76.9	97.3	2.7
	50	45.3	93.5	6.5
12	10	97.9	98.1	1.9
	20	96.8	97.0	3.0
	30	89.5	97.7	2.3
	40	70.0	97.7	2.3
	50	31.3	90.9	9.1

Table III. Ascorbic Acid Retention in Single-Strength Orange Juice vs. Single-Strength Grapefruit Juice after 12 Weeks of Storage at Various Temperatures

temp, °C	grapefruit juice, % reten	orange juice,[a] % reten
10	98.7	98.5
20	96.5	96.3 (21 °C)
30	89.9	92.0 (29.4 °C)
40	70.3	70.3 (40.6 °C)
50	29.3	5.6 (49 °C)

[a] Data from Nagy and Smoot (1977).

Figure 1 shows these two parallel pathways for AA degradation. In the first pathway (aerobic), the initial breakdown product (DHA) is antiscorbutically active; however, the subsequent breakdown products are not active. In the second pathway (anaerobic), no breakdown products are active. Thus, the pathway of degradation may have an effect on antiscorbutic activity.

Table II shows that increases in storage temperature and time caused a decrease in AA retention. The percentages of AA retained in SSGJ after 12 weeks of storage at different temperatures compare favorably with those found for canned single-strength orange juice (SSOJ) (Table III).

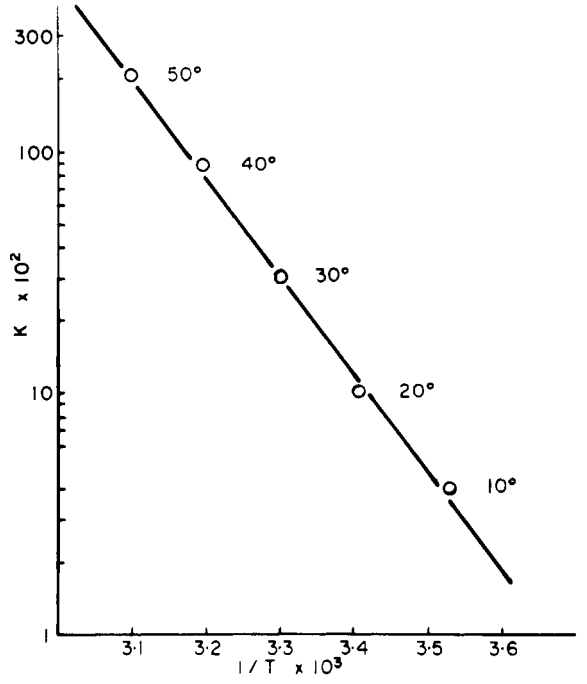

Figure 2. Arrhenius plot of log rate K [mg of vitamin C loss (100 mL of juice^{-1} week^{-1}] vs. reciprocal of absolute storage temperature. Regression analysis of data shows E_a = 18.2 kcal/mol and Q_{10} = 2.7.

Table IV. Zero- and First-Order Reactions for Vitamin C Degradation at Five Storage Temperatures

temp, °C	zero order ($a = b + cx$)			first order ($a = b + c \ln x$)		
	intercept	rate[a]	R^2	intercept	rate[a]	R^2
10	35.01	0.04	0.99	34.79	0.04	0.63
20	34.96	0.11	0.95	34.33	0.11	0.69
30	35.19	0.30	0.98	33.43	0.26	0.52
40	35.17	0.88	1.00	30.00	0.81	0.60
50	33.96	2.05	0.99	21.92	2.08	0.71

[a] Loss of vitamin C as mg of vitamin C (100 mL)$^{-1}$ week^{-1}.

The exception to this was the AA retention in juice stored at 50 °C. At this temperature SSGJ retains more AA than is retained in SSOJ under similar conditions. Higher percent retentions of AA in SSGJ than in SSOJ at high storage temperatures and different storage periods have been reported (Lamb, 1946). As expected, the rate of AA degradation in SSGJ increased with temperature. To quantitate this increase, the Q_{10} of the reaction (the increase in rate for a 10 °C rise in temperature) was calculated from the increase in AA loss of [mg of AA (100 mL of juice)$^{-1}$ week^{-1}]. An Arrhenius plot of log K (rate constant) vs. the reciprocal of absolute storage temperature is shown in Figure 2. The energy of activations (E_a) for ascorbic acid degradation in SSGJ can be calculated by the slope method

$$\text{slope} = \frac{-E_a}{(2.3)(1.99)}$$

Regression analysis of the slope in Figure 2 yielded an E_a = 18.2 kcal/mol. Using the formula method of Waletzko and Labuza (1976), that is

$$\log Q_{10} = \frac{2.2E_a}{(T)(T + 10)}$$

the results showed that a temperature rise of 10 °C caused

Table V. Polynomial Regression Expressions for Ascorbic Acid (AA) Degradation at Various Storage Temperatures

temp, °C	polynomial expression[a]
10	$\bar{y} = 99.24 - 0.34u$
20	$\bar{y} = 97.66 - 0.92u + 0.17u^2$
30	$\bar{y} = 95.88 - 2.54u - 0.24u^2$
40	$\bar{y} = 86.24 - 7.52u - 1.81u^2 + 0.41u^4$
50	$\bar{y} = 58.78 - 17.60u + 1.49u^2$

[a] $u = (x - 6)/3$, x = weeks of storage, $\bar{y}$ = estimated retention of vitamin C.

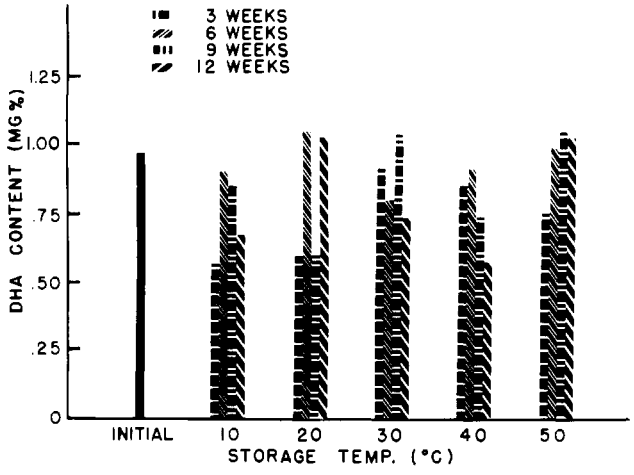

Figure 3. Effects of storage time and temperature on dehydroascorbic acid (DHA) contents in canned single-strength grapefruit juice.

an increase in the reaction rate of about 2.5 to 3.0.

Reaction rates were calculated for vitamin C degradation, assuming either a zero or first-order reaction. The results (Table IV) show good correlation with zero-order reactions. The correlations with first-order reactions were not as good. The zero point intercept should be at 35.02 (to correspond to the initial level of vitamin C in the samples). The deviation from this in the first-order reactions was large. Even in the zero-order reactions the variation was noticeable, particularly in the reaction for 50 °C juice. This may be caused by an initial rapid degradation caused by residual oxygen in the headspace and dissolved in the juice (Nagy and Smoot, 1977). This may cause errors in predicting vitamin C loss using zero-order reactions at high temperatures for short storage times. For this reason the data was fit by orthogonal polynomial regression analyses (Hicks, 1973). The analyses were calculated on a percent loss rather than a milligram loss. These polynomial regression expressions can be used to calculate relative AA percent retention in canned SSGJ samples by entering the value for the length of storage into the rate expression for that storage temperature (Table V).

DHA values were calculated for the juices, and average values ranged from 0.58 to 1.06 mg % (Figure 3). Analyses showed DHA content to be unaffected by storage time or storage temperature. The sum of the DHA and AA values, the TAVC values, showed the same pattern of retention loss as AA alone. The percentages of TAVC due to AA and DHA and retentions of TAVC at various temperatures and lengths of storage are listed in Table II. Only 1.6–3.1% of the total antiscorbutic activity, during any storage time, was attributable to the DHA content. The only exception to this was juice stored at 50 °C for more than 3 weeks. The decrease of AA in that juice was so rapid and the final retention so small that the DHA content (although no

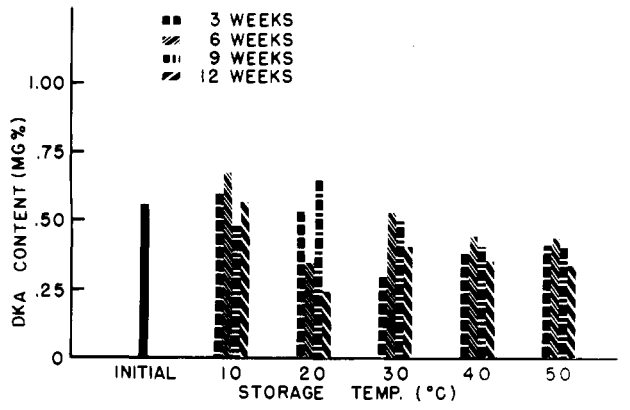

Figure 4. Effects of storage time and temperature on diketogulonic acid (DKA) contents in canned single-strength grapefruit juice.

larger in mg %) comprised a higher percentage of the total vitamin C activity than observed for the other storage temperatures.

DKA content (a measure of DHA breakdown) also showed no significant change during this study (Figure 4). The average values ranged from 0.24 to 0.67 mg %.

The lack of significant changes in DHA and DKA values during this storage study indicates the possibility of little or no AA degradation by the aerobic pathway. Breakdown of AA by the second pathway (anaerobic) should not be unexpected. Free oxygen (in the headspace and dissolved in the juice) is consumed by chemical reactions within the first two weeks of storage (Kefford et al., 1959; Nagy and Smoot, 1977); AA degradations must from then on proceed anaerobically. Whether anaerobically or aerobically, vitamin C degradation forms furfural as its major degradation product (Huelin, 1953). Buildup of furfural has been reported in both canned SSGJ and SSOJ during high-temperature storage (Nagy et al., 1972; Nagy and Randall, 1973). A high sample correlation coefficient ($r = 0.79$) has been recorded for furfural and vitamin C in SSOJ (Nagy and Dinsmore, 1974). Tannebaum (1976) speculated that AA might degrade anaerobically (also to furfural) by a pathway, different from that shown in Figure 1. This pathway is not favored by the authors because of the speculative formation of DKA. Vitamin C degrades to form furfural but the intermediate steps in the anaerobic pathway are still highly speculative.

The degradation rate of AA was basically explained by a zero-order reaction at the temperatures studied. Our data agreed with the work of Kefford et al. (1959) which provided evidence that the relation between log ascorbic acid content and storage temperature at specific storage temperatures may not be linear. AA retention in stored SSGJ could be predicted through application of orthogonal polynomial formulas. Except at extremely high storage temperatures (50 °C or higher) for longer than 12 weeks, TAVC in canned SSGJ could be determined, with only slight error, from AA data only. Cool storage is essential for retention of vitamin C activity in canned SSGJ. High-temperature storage, even for short periods, will cause extensive loss of vitamin C activity.

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3′-Hydroxyzearalenones, Two New Metabolites Produced by *Fusarium roseum*

Sadanand V. Pathre, Stuart W. Fenton, and Chester J. Mirocha*

Two new metabolites related to zearalenone [6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcylic acid lactone] were isolated from the rice cultures of *Fusarium roseum* and were shown to be diastereomeric 3′-hydroxyzearalenones [6-(3,10-dihydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcylic acid lactone]. The accumulation of 3′-hydroxyzearalenones as the culture ages suggests that these hydroxy derivatives are the product of zearalenone metabolism by *F. roseum*.

Fusarium roseum under certain conditions of moisture and temperature infects maize and produces a mycotoxin called zearalenone (Urry et al., 1966; Stob et al., 1962) [6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcylic acid lactone]. When such corn is fed to swine, it causes the estrogenic syndrome which involves primarily the genital system (Christensen et al., 1965; Mirocha et al., 1971; Mirocha and Christensen, 1974).

During the search on new metabolites related to zearalenone, Mirocha et al. (1974) reported the isolation of two derivatives, namely, F-5-3 and F-5-4, which were thought to be 3′-hydroxyzearalenones. However, Jackson et al. (1974) revised the structures of F-5-3 and F-5-4 as diastereomeric 8′-hydroxyzearalenones (Bolliger and Tamm, 1972). Further, Mirocha et al. (1971) reported that a significant estrogenic activity was found in a number of components of *Fusarium* culture extracts. Some of those components were more polar than 8′-hydroxyzearalenones. Steele et al. (1976) reported the isolation of 6′, 8′-dihydroxyzearalene, presumably produced through the metabolism of zearalenone by *Fusarium*. We isolated two minor metabolites; their isolation and characterization are the subject of this paper.

RESULTS AND DISCUSSION

The extensive chromatographic separation of extracts of 2.5 kg of rice cultures of *Fusarium roseum* yielded 30 mg of crystalline F-5-3, (8′*R*,10′*S*)-8′-hydroxyzearalenone (II) and 40 mg of F-5-4, (8′*S*,10′*S*)-8′-hydroxyzearalenone (III). The UV, IR, and mass spectra of these compounds were identical with those of the authentic samples isolated by Jackson et al. (1974).

Chromatographic fractions containing a fluorescent component more polar than F-5-3 were pooled and were purified by preparative thin-layer chromatography on silica gel repeatedly developed in chloroform-absolute ethanol (97:3, v/v). The successive chromatographic separation by preparative thin-layer chromatography yielded 6 mg of a pure compound. The ^1H and ^{13}C NMR spectra in acetone-d_6 indicated that the unknown fluorescent compound was related to zearalenone. The compound appeared to be homogeneous (single component) on TLC when developed in several different solvent systems. However, it was resolved into two bands at R_f 0.33 and 0.28 when developed in chloroform–absolute ethanol (95:5, v/v). Both components yielded mass spectra identical with respect to fragmentation pattern. The component at R_f 0.28 was designated as F-5-1 and that at R_f 0.33 as F-5-2. Amounts of F-5-1 and F-5-2 after separation were <0.4 and <2.0 mg, respectively.

The high-resolution mass spectra established the elemental composition of $C_{18}H_{22}O_6$ and molecular weight as 334 for both compounds compared to $C_{18}H_{22}O_5$ and 318 for zearalenone.

The trimethylsilyl ethers of F-5-1 and F-5-2 were inseparable by gas–liquid chromatography on 3% OV-1, OV-17, or QF-1 columns. However, they gave identical mass spectra with molecular ion at m/e^+ 550 compared to a molecular ion at m/e^+ 462 for zearalenone and at m/e^+

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