Other flavor-related components detected in snuff tobacco at exceptionally high concentrations included geraniol, benzyl alcohol, 1,8-cineole, phenylethanol, and syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde). In one of the commercial brands, geraniol was quantified at 0.6 μg/g of tobacco (dry weight) and syringaldehyde at 49 μg/g of tobacco (dry weight). We also detected menthol, isomenthol, and menthone in some of the commercial snuff brands.

Several of the compounds identified in this study (phenol, methyl salicylate, benzyl benzoate), which appear to be additives to commercial snuff tobaccos, are known irritants. These constituents in commercial snuff tobacco can potentially influence the genotoxic activity of the identified tobacco carcinogens, N′-nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, and benzo[a]pyrene (Hoffmann et al., 1986, 1987). This possibility is currently being evaluated.

ACKNOWLEDGMENT

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Registry No. Benzyl alcohol, 100-51-6; phenol, 108-95-2; cineole, 470-82-6; α-terpineol, 98-55-5; acetylpyridine, 350-03-8; phenylethanol, 60-12-8; menthol, 89-78-1; neomenthol, 491-01-0; methyl salicylate, 119-36-8; ethyl salicylate, 118-61-6; β-citronellol, 106-22-9; geraniol, 106-24-1; acetovanillone, 498-02-2; 4-hydroxyacetophenone, 99-93-4; syringaldehyde, 134-96-3; benzyl benzoate, 120-51-4.

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Changes in Composition of Volatile Components in Aseptically Packaged Orange Juice during Storage

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A procedure involving low-pressure distillation and capillary gas chromatography of the distillate was used to monitor 29 volatile components of aseptically packaged orange juice during 8 months of storage at 21 and 26 °C. A gradual decrease in several flavor components, 1-penten-3-one, hexanal, ethyl butyrate, octanal, neral, and geranial, and an increase in two undesirable components, furfural and α-terpineol, were observed in addition to other changes. After 2-month storage, an experienced taste panel found a significant difference in stored juices compared to a control sample and a significant preference for the starting control juice. Continued decreases in oil content and ascorbic acid were noted during the 8-month storage period.

Aseptically packaged fruit juices and fruit juice drinks are the fastest growing segment of the fruit beverage industry (Tillotson, 1984). Some of the products have relatively short shelf lives because they undergo flavor changes at supermarket shelf temperatures (21 °C). These changes are particularly noticeable in 100% orange juice that has been packaged aseptically; the flavor changes inhibit the full market potential of this product. There is a need for analytical techniques to provide information on the specific flavor and compositional changes that aseptically packaged fruit juices undergo during storage. From this information, methods can be proposed for inhibiting or retarding such detrimental changes.

Limited studies have been carried out that show changes in a few specific volatile components during storage of orange juice packaged aseptically. Durr et al. (1981) found

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a linear increase in α-terpineol with storage and an increase in its rate of formation as storage temperature increased. They also found that significant decreases in *d*-limonene occurred during the first 6 days of storage and then leveled off. Marshall et al. (1985) found a similar decrease in limonene content during storage. Decreases in desirable flavor components neral, geranial, octanal, and decanal were found also by Durr et al. (1981). Nonenzymatic browning was correlated with flavor and compositional changes by several workers (Potter et al., 1985; Mannheim, 1985; Mannheim et al., 1987; Marcy and Graumlich, 1982; Graumlich et al., 1986). Marcy and Graumlich (1982) showed the exclusion of oxygen to be important in retarding browning development.

The present study uses the application of a technique developed earlier (Moshonas and Shaw, 1987) to monitor changes in volatile constituents in aseptically packaged orange juice and relates these changes to limited taste panel studies.

EXPERIMENTAL SECTION

Commercially prepared samples of orange juice packaged aseptically in 250-mL flexible multilayer cartons were obtained the same day they were packaged. The shelf life code dated on the packages was 6 months. One sample was analyzed immediately, and the remaining samples were placed in storage at −21 (control), +21, and +26 °C. Samples were removed at 2-month intervals over the next 8 months for analysis.

Recoverable oil was determined by the method of Scott and Veldhuis (1966) and ascorbic acid by the method of Spaeth et al. (1962). Volatile constituents were monitored by a modification of a procedure described earlier (Moshonas and Shaw, 1987). In the current study, a distillate representing 50% of the total volume of each juice sample was collected by distillation at <60 °C (150 mmHg). Six-microliter portions of each distillate were injected into a Hewlett-Packard Model 5880A gas chromatograph (GC) equipped with a flame ionization detector and a Hewlett-Packard 50-m, wide-bore (0.31–0.32-mm i.d.) capillary fused silica cross-linked 5% phenylmethyl silicone column under GC conditions described earlier (Moshonas and Shaw, 1987). Peaks were identified by comparison of retention times with times of peaks enriched with authentic standards and with times determined earlier in fresh orange juice (Moshonas and Shaw, 1987). Quantitative estimates shown in Table I were made as described earlier (Moshonas and Shaw, 1987). Coefficients of variation for triplicate runs on the starting juice were 11% or less for peaks of 0.4 ppm or more and 43% or less for peaks of 0.2–0.3 ppm.

Flavor panel studies were conducted with an experienced 12-member panel. Each panelist was given two presentations for a total of 24 judgements. Triangle and paired flavor tests used were described by Boggs and Hanson (1949). In triangle tests, each presentation consisted of three samples, two of which were identical. Panelists were asked to indicate which sample was different. In paired comparison tests, panelists were asked to indicate which sample they preferred. Tests compared control juice versus juices stored at 21 and 26 °C for 2 months.

RESULTS AND DISCUSSION

Aseptically packaged orange juice stamped with a 6-month expiration date was used in this study. A distillate from the aseptically packaged juice sample was analyzed by GC for volatile components at 2-month intervals over an 8-month period. The GC profiles of volatile components, which were determined initially and at 2- and 4-month storage at 21 °C are shown in Figure 1. A GC profile on distillate from starting control juice showed at least 45 individual peaks are present in the GC curve. Of these peaks 29 have been identified, and they are listed in Table I along with quantitative estimates for most of them in freshly processed aseptically packaged juices. All but one of the identified components have been reported in aqueous orange essence (Shaw, 1977), a flavor fraction added to juice during reconstitution to prepare single-strength juice for packaging (Johnson and Vora, 1983). Peak 18 is a mixture of two components unresolved by this nonpolar GC column; a polar Carbowax column will resolve this mixture (Moshonas and Shaw, 1987). The one tentatively identified peak not reported earlier as a volatile citrus component was an acetal, 2,2-diethoxypropane (peak 14 in Figure 1 and Table I). This acetal can be formed from two other components, ethanol and acetone, under acidic conditions. Such acetals are probably artifacts formed during the preparation of aqueous essence (Nursten, 1970).

Table I. Volatile Components of Aseptically Packaged Orange Juice

peak no.	compound	amt,[a] ppm
1	methanol	ND[b]
2	acetaldehyde	2.6
3	ethanol	ND
4	acetone	1.4
5	1-propanol	1.0
6	ethyl acetate	2.0
7	2-methylpropanol	0.4
8	1-butanol	0.3
9	1-penten-3-one	0.4
10	2-pentanol	0.2
11	methyl butyrate	0.2
12	3-methyl-1-butanol	0.7
13	2-methyl-1-butanol	0.3
14	2,2-diethoxypropane[c]	0.2
15	ethyl butyrate	0.9
16	1-hexanal	0.5
17	furfural	0.0
18	*trans*-2-hexenal + *cis*-3-hexen-1-ol	0.6
19	*trans*-2-hexenol	0.3
20	α-pinene	0.2
21	myrcene	ND
22	octanal	1.0
23	limonene	ND
24	linalool	11
25	terpinen-4-ol	1.5
26	α-terpineol	1.9
27	neral	1.7
28	geranial	1.7
29	valencene	0.3

[a] Calculated by a procedure reported earlier (Moshonas and Shaw, 1987). [b] ND = not determined. [c] Tentative identification.

Of the identified components listed in Table I 17 underwent notable quantitative changes during storage at both temperatures. Relative changes in amounts of these components during storage at 21 °C are shown in Table II. The values in this table are from single or duplicate runs, and thus statistical analyses were not possible. The largest peak, ethanol, was considered a constant throughout the study and was thus used as an internal standard to determine the relative changes shown in this table. The relative concentration for each component in the starting juice sample was considered 1.0 unless the amount was too small to quantify or the compound was undetected (furfural). By this technique, relative changes for individual components could be tabulated during the storage study. Storage at 26 °C caused the same changes to occur as those observed at 21 °C, but at a faster rate. Although asepti-

Table II. Changes with Storage in Relative Amounts of Selected Volatile Juice Components.

component	storage time, at 21 °C, months					
	0	2	4	6	8	10
		Decreasing				
1-butanol	1.0	0.9	0.9	0.9	0.6	0.6
ethyl butyrate	1.0	0.7	1.1	0.6	0.8	0.6
geranial	1.0	ND[a]	ND	ND	tr[b]	ND
1-hexanal	1.0	1.0	0.9	0.5	0.5	0.5
trans-2-hexenal + *cis*-3-hexenol	1.0	0.8	0.4	0.3	tr	tr
trans-2-hexenol	1.0	1.0	0.8	0.6	tr	tr
neral	1.0	0.2	ND	ND	ND	ND
octanol	1.0	0.5	0.5	0.3	0.2	0.2
1-penten-3-one	1.0	tr	tr	tr	tr	tr
		Increasing				
ethyl acetate	1.0	1.5	2.9	3.1	3.3	3.4
furfural	ND	1.0	2.3	6.8	5.8	8.3
α-terpineol	1.0	2.9	3.4	4.2	4.3	4.9
		Variable				
acetone	1.0	0.8	1.6	1.9	2.0	1.8
2,2-diethoxypropane	1.0	4.2	6.2	8.4	6.8	7.2
limonene	1.0	1.3	5.5	2.9	2.0	2.2
myrcene	1.0	1.2	7.0	3.6	2.2	2.7

[a] ND = not determined. [b] tr = trace.

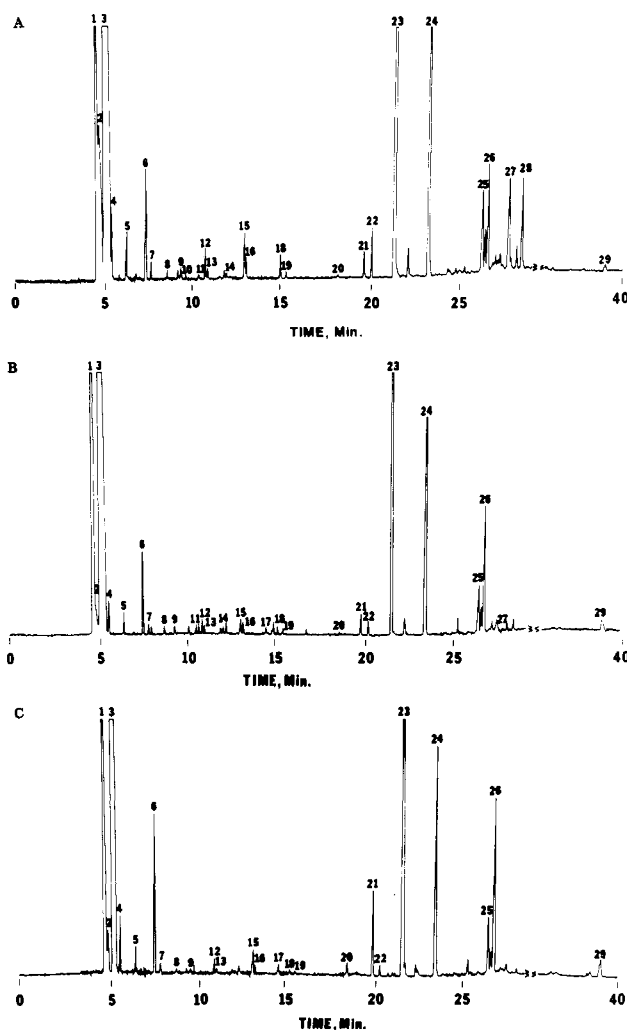

Figure 1. GC profiles of volatile components in aseptically packaged orange juice after (A) 0 months (control), (B) 2 months at 21 °C, and (C) 4 months at 21 °C storage.

cally packaged juices are often stored at 21 °C on supermarket shelves, 26 °C probably better reflects the average storage temperatures once the product is purchased by the consumer.

Several of the volatile flavor components showed marked decreases during storage (Table II). These included components that are known to contribute to fruit flavors: ethyl butyrate, geranial, hexanal, *trans*-2-hexenal, neral, octanal, 1-penten-3-one (Arctander, 1969; Ahmed et al., 1978). Acetaldehyde is another important flavor compound in orange juice whose quantity appeared to decrease during storage. Since acetaldehyde was incompletely resolved from a much larger methanol peak, its loss during storage was not easy to follow with this technique.

The two major components of orange oil, limonene and myrcene, were present. Finding these oil components in varying quantities indicated incomplete solubility in the aqueous distillate. Measurement of total recoverable oil (see below) afforded more consistent results.

The identified components that continued to increase during storage were ethyl acetate, furfural, and α-terpineol. Passy (1983) found several volatile solvents including ethyl acetate to increase in an aseptically packaged fruity soft drink during storage. He found the increase in ethyl acetate to be due to migration of the solvent from laminated containers, and it varied widely among individual packages. In our study, no examination of unused laminated containers for volatile components was possible. Furfural, which is known to increase in other orange juice products, has been used as an index for storage abuse in citrus juices. Its concentration is too low to make it a direct contributor to off-flavor, however (Nagy and Dinsmore, 1974). The α-terpineol increased progressively at 21 °C and was at almost 5 times its initial level after 5 months. In one sample of aseptically packed orange juice that had been stored on a supermarket shelf for 7 months past its expiration date, α-terpineol was the major volatile component, and it was present in that sample at about 10 times the amount of limonene detected. The results found in this study involving α-terpineol formation in aseptically packaged orange juice parallel those reported earlier by Durr et al. (1981). α-Terpineol is probably formed from limonene by hydration under the acidic juice conditions (Slater and Watkins, 1964). Tatum et al. (1975) found α-terpineol to be an off-flavor storage product in canned orange juice. It contributed a stale, musty, or pinelike aroma to the juice.

Four identified juice components showed varying amounts present during storage (Table II). Variable quantities of the two major oil components, limonene and

Table III. Oil and Ascorbic Acid Contents in Stored Aseptically Packed Orange Juice

storage time, months	oil level,[a] %		ascorbic acid,[a] mg/100 g	
	21 °C	26 °C	21 °C	26 °C
0 (control)	0.0176	0.0176	43.6	43.6
2	0.0093	0.0089	35.2	33.5
4	0.0085	0.0087	34.8	32.8
6	0.0079	0.0080	32.7	29.4
8	0.0065	0.0065	25.2	19.9

[a] Average of duplicate analyses.

myrcene, possibly indicated incomplete water solubility during the distillation procedure. The total oil content (see below) decreased steadily during the storage study, as had been reported earlier (Durr et al., 1981).

Acetone content was variable, but generally increased during storage. 2,2-Diethoxypropane was probably an artifact formed from acetone and ethanol as described above, and its variability and general increased content was not surprising.

A flavor panel was used to evaluate juices stored at 21 and 26 °C for 2 months. The panel determined a significant difference at the 99.9% confidence level in juice stored at either 21 or 26 °C when compared to juice from the original sample kept at −21 °C for the same 2-month period (control sample). There was a significant preference at the 99% confidence level in a paired comparison test for the control sample over juice stored at either 21 or 26 °C. Most panel members stated that an aged or stale flavor, rather than a heated or processed flavor, developed in the juices stored at 21 and 26 °C. Development of a stale flavor in orange juice has been attributed to the loss of volatile esters (Blair et al., 1952) and to the buildup of α-terpineol during storage (Tatum et al., 1975). Both of these changes were observed in this study. Since the stored juices were easily differentiated from the control sample after 2-month storage, flavor tests were not conducted on juices stored for longer periods.

Recoverable oil and ascorbic acid levels were determined in samples of juice stored at 21 and 26 °C at 2-month periods during this study (Table III). The oil level dropped to about half the original value during the first 2 months and then continued to drop more slowly over the next 6 months of storage at both 21 and 26 °C. Other workers have reported losses in limonene primarily due to absorption by the polymeric package liner, although a small amount of limonene was converted to α-terpineol (Durr et al., 1981; Marshall et al., 1985). In one study, the rate of limonene absorption was directly related to the thickness of the polypropylene liner in the aseptic package (Marshall et al., 1985). A gradual loss in ascorbic acid content during storage at both 21 and 26 °C was noted also, as shown in Table II. These findings were similar to losses in ascorbic acid during storage of aseptically packaged orange juice reported earlier (Wilson and Shaw, 1987).

This study demonstrates a useful technique for estimating changes in volatile constituents present in aseptically packaged orange juice. From these quantitative estimates, decreases in several compounds important to good orange flavor and increases in two undesirable flavor components during storage were noted. This analytical technique is rapid, minimizes artifact formation, and eliminates losses through solvent extraction. The technique should be applicable to the study of flavor changes that take place during processing and storage of other fruit juices and drinks as well.

Registry No. Ascorbic acid, 50-81-7; methanol, 67-56-1; acetaldehyde, 75-07-0; ethanol, 64-17-5; acetone, 67-64-1; 1-propanol, 71-23-8; ethyl acetate, 141-78-6; 2-methylpropanol, 78-83-1; 1-butanol, 71-36-3; 1-penten-3-one, 1629-58-9; 2-pentanol, 6032-29-7; methyl butyrate, 623-42-7; 3-methyl-1-butanol, 123-51-3; 2-methyl-1-butanol, 137-32-6; 2,2-diethoxypropane, 126-84-1; ethyl butyrate, 105-54-4; 1-hexanal, 66-25-1; furfural, 98-01-1; *trans*-2-hexenal, 6728-26-3; *cis*-3-hexen-1-ol, 928-96-1; *trans*-2-hexenol, 928-95-0; α-pinene, 80-56-8; myrcene, 123-35-3; octanal, 124-13-0; limonene, 138-86-3; linalool, 78-70-6; terpinen-4-ol, 562-74-3; α-terineol, 98-55-5; neral, 106-26-3; geranial, 141-27-5; valencene, 4630-07-3.

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Sulfhydryl Group/Disulfide Bond Interchange Reactions during Heat-Induced Gelation of Whey Protein Isolate

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The kinetics of reaction between the SH group of β-lactoglobulin and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) was used to detect the SH/S–S interchange reactions taking place when dispersions of whey protein isolate (1 or 9% protein) were heated at 85 °C at pH 7.5 or 2.5. The method is based on the assumption that the reactivity of the SH^{121} group adjacent to the $S–S^{106–119}$ bond (native state) is low in the presence of sodium dodecyl sulfate (SDS). However, the new SH group formed in position 66 or 160 through an SH/S–S interchange reaction reacts rapidly with DTNB in the presence of SDS. The data of reaction kinetics were compared to those of protein solubility, gel texture, and SDS–polyacrylamide gel electrophoresis. Heating a 9% protein dispersion caused (1) the formation of a highly elastic gel at pH 7.5 [intermolecular S–S bonds due essentially to SH/S–S interchange reactions are predominant in the gel network and partly responsible for the high elasticity] and (2) the formation of a nonelastic gel at pH 2.5.

It is known that disulfide (S–S) bonds and sulfhydryl (SH) groups play an important role in the heat-induced gelation of proteins. Covalent cross-linking of protein molecules can be brought by SH oxidation into S–S bonds and/or by SH-induced S–S interchange reactions. This has been reported by Yasuda et al. (1986) for bovine serum albumin (BSA), by Jiang et al. (1986) for fish proteins, by Mori et al. (1982), Utsumi and Kinsella (1985a,b), Mori et al. (1986), and Shimada and Cheftel (1988a) for soy proteins, by Shimada and Matsushita (1980) and Hayakawa and Nakai (1985) for egg albumin, by Beveridge et al. (1984) for egg albumin and whey protein concentrate, and by Schmidt et al. (1978, 1979), Dunkerley and Zadow (1984), and To et al. (1985) for whey protein concentrate. However, none of these studies has indicated whether intermolecular S–S bonds in the network of protein gels depend mainly on SH oxidation into additional S–S bonds or on SH/S–S interchange reactions.

The changes in SH group/S–S bond contents have been recently investigated in heat-induced gels of whey protein isolate (WPI) (Shimada and Cheftel, 1988b). It was observed that the rate of reaction between SH groups and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (in a Tris buffer containing 8 M urea and 0.5% sodium dodecyl sulfate) was lower for unheated WPI than for heat-processed WPI. It has also been demonstrated that β-lactoglobulin (β-LG), the major protein of WPI, reacts about 150 times slower with *N*-ethylmaleimide (NEM) in 1% sodium dodecyl sulfate (SDS) than in 8 M urea (Franklin and Leslie, 1968). These results lead to the hypothesis that SDS may inhibit the reaction between the SH group of native β-LG and DTNB and that the inhibition by SDS decreases after heat processing of β-LG.

In the present study, it is attempted to determine changes in the contents of slow-reacting (with DTNB) SH groups (mainly the SH group in position 121 adjacent to the S–S bond between Cys^{106} and Cys^{119}; native β-LG) as a function of protein concentration and of pH during the heating of WPI dispersions. The method takes advantage of differences in kinetics and is based on the assumption that the SH^{121} group of β-LG reacts slowly with DTNB in the presence of SDS as compared to SH groups in other positions, especially those formed through SH/S–S interchange reactions. Correlations between the texture of WPI gels and SH/S–S interchange reactions have also been investigated.

MATERIALS AND METHODS

Materials. Bovine β-lactoglobulin-1 (β-LG-1; lyophilized, Lot No. 52F-8035) and bovine β-lactoglobulin-2 (β-LG-2; 3× crystallized and lyophilized; Lot No. 36F-8085) were obtained from Sigma Chemical Co., St. Louis. Both β-LG contained genetic variants A and B, and β-LG-1 also contained about 2% NaCl.

Whey protein isolate (WPI) was obtained as indicated previously (Shimada and Cheftel, 1988b). It contained about 72 g of native β-LG and 5 g of native α-lactalbumin (α-LA) per 100 g of protein.

Heat Treatment of WPI Dispersions and Preparation of Gels. The aqueous dispersions of WPI (1 or 9% protein) were adjusted to pH 7.5 or 2.5 with 6 NaOH or 6 N HCl, treated for partial deaeration (to avoid air bubbles in the subsequent gels) with a water pump for 2 min, and placed in glass tubes (2.2-cm i.d. × 4.5-cm height) with tightly closed stoppers. The tubes were heated at 85 °C for varying periods of time (0–45 min) in a water bath and then cooled rapidly in ice water. After being allowed to

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