Table III. General Composition of Pectic Substances Isolated from Golden Delicious and Delaware Juices

Yield, mg/100 ml of juice	Golden Delicious 89.2	Delaware 675
Polysaccharide, %	67.4	11.9
Pectin, %	58.5	8.5
Neutral sugar, %	8.9	3.4
Protein, %	4.4	3.2
Ash, %	2.7	28.7
Unknown components, %	25.5	56.2

Such variation is related to the susceptibility of grape juice to enzymatic clarification. It seems likely that the boundary of susceptibility of fruit juice to pectin lyase and *endo*-polygalacturonase may exhibit about 55% of esterification of pectin in the juice.

The present study could not make it clear what kind of component in the crude enzyme may be a promoting factor and why it is necessary for clarifying only grape juice. Great difference was not found, as shown in Table II, in the pectin content of pectic substances isolated from apple and grape juice. However, it is not certain whether neutral sugar detected in these substances is bounded to pectin as a constituent or not, since these pectic substances were isolated as only 75% ethanol-insoluble materials and were not purified.

In general composition, these materials isolated from grape juice (Delaware), as compared with those from apple juice, were low in polysaccharide content but contained a very high percentage of ash and unknown components (Table III). We suggest that the elucidation of the nature and the functional role of the promoting factor in clarifying grape juice is not likely to be a simple matter.

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Use of Furfural Content as an Index of Storage Temperature Abuse in Commercially Processed Orange Juice

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Furfural was measured over a 16-week period in canned orange juice and orange juice packed in glass at 5, 10, 16, 21, and 30°. Examination of canned juice showed that for every 5° temperature rise in storage there was an approximate doubling of the furfural content. Organoleptic evaluation showed that when the level of furfural exceeded 55 μg/l. of juice, a taste panel observed a difference in flavor in comparison to controls at a significance of $p < 0.001$. Furfural, *per se*, was not the component responsible for the flavor change, but its levels appeared to parallel closely the extent of comparative flavor differences.

Commercially processed orange juice is highly susceptible to flavor change when stored at warm temperatures and prolonged storage periods. Over the past 30 years, many chemical changes occurring in adversely-stored citrus juices have been reported by scientists working in the citrus industry. Nolte and von Loesecke (1940) showed that temperature-aged Valencia orange juice differed from fresh juice by increased acidity, increased saponification and peroxide values, and by the presence of carbonyl compounds. Huskins *et al.* (1952) investigated the change in lipid composition of orange juice at 22° for 2 years and found that phospholipid phosphorus content decreased to one-tenth its original value, lipid nitrogen decreased to one-fifth its original value, and lipid choline had completely disappeared. The relationship of lipid degradation to flavor change has been further substantiated by the works of Curl and Veldhuis (1947), Swift (1951), and Nagy and Nordby (1970).

Although many chemical tests (Vandercook, 1970) are available to indicate temperature abuse of citrus juice, there has remained a need for a simple analysis which

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would function as an index of storage experience. Of the various known compounds formed when citrus juice deteriorates (peroxides, aldehydes, ketones, oxygenated terpenes, and furfural) the last of these has the most satisfactory features for the desired analysis. The furfural content of freshly processed citrus juice is essentially zero, whereas large amounts have been recognized in temperature-aged juice by Kirchner and Miller (1957), Rymal *et al.* (1968), and Dinsmore and Nagy (1971). The use of furfural content as an index of temperature abuse has not been extensively employed because of the lack of a sensitive and reliable procedure for its determination in citrus juices. This problem has been removed by the recent development of a method by Dinsmore and Nagy (1972) which possesses the accuracy and sensitivity to detect furfural in commercial citrus juices down to a level of 5 ppb.

The purpose of the following study is to determine the mode and extent of build-up of furfural in commercially-processed orange juice stored in both can and glass containers at five storage temperatures. In addition, organoleptic tests are conducted simultaneously with furfural analysis to determine relationships among storage times, storage temperatures, furfural content and flavor changes.

MATERIALS AND METHODS

Orange Juice Samples and Storage. Commercially-processed orange juice from the midseason orange crop was obtained from Citrus World, Inc., Lake Wales, Fla. Fifteen cases of 46-oz single strength juice in cans and 15 cases of 32-oz single strength juice in glass were obtained directly from the assembly line. The samples were, in turn, divided equally and placed in five storage lockers possessing the following temperatures: 5, 10, 16, 21, and 30°. Samples were taken from the lockers weekly for the first 4 weeks and thereafter biweekly for the next 12 weeks. Samples were subjected to furfural determination and organoleptic evaluation.

Furfural Determination. The furfural content of orange juice was measured according to the method of Dinsmore and Nagy (1972). In brief, the method consisted of distilling 200 ml of single-strength orange juice at a distillation rate of *ca.* 3 ml/min until 10 ml was collected with the apparatus described by Scott and Veldhuis (1966). Two milliliters was taken from the 10-ml distillate and to this was added 2 ml of 95% ethyl alcohol and 1 ml of aniline reagent (5 ml of freshly distilled aniline made up to 50 ml with glacial acetic acid). The color which developed after 15 min was measured at 515 nm in a Bausch & Lomb Spectronic 20.

Organoleptic Evaluation. All flavor evaluations were made with the triangular comparison test discussed by Boggs and Hanson (1949), Byer and Abrams (1953), and Berry *et al.* (1967). In these tests, juice stored slightly above freezing or 4–5° was used as control. Employing 4–5° stored juice as reference was based upon the observation of Dinsmore and Nagy (1972) that flavor change in orange juice stored at 5° or below does not take place for approximately 1½ years. This control was compared to juice stored at 10, 16, 21, and 30° over a 16-week period. In the triangular test, 12 experienced tasters were each given two presentations. All judges were trained to differentiate juice through both taste and smell. The statistical significance of this test was recorded according to the procedure of Roessler *et al.* (1948).

RESULTS AND DISCUSSION

Freshly processed citrus juice has been shown by Rymal *et al.* (1968) and by Dinsmore and Nagy (1971) to contain virtually zero furfural levels. The canned and glass-packed commercial orange juice used in this study had an initial furfural level of 8 μg per l. of juice, which for all practical

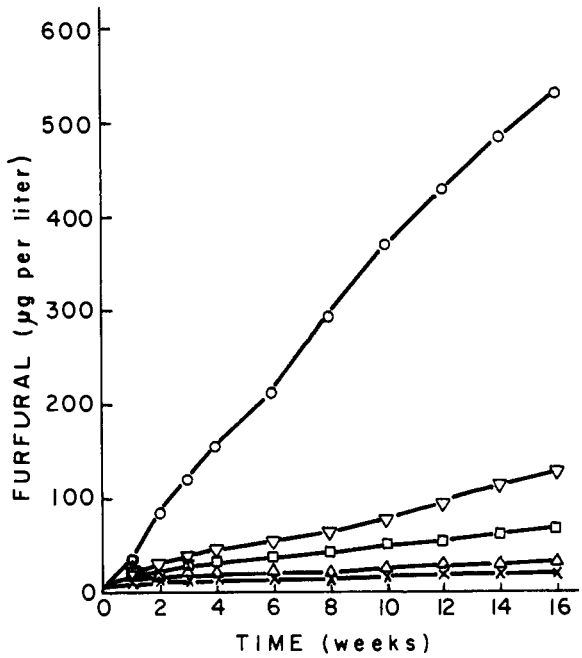

Figure 1. Increase in furfural content in canned orange juice over a 16-week period at 5° (X), 10° (△), 16° (□), 21° (▽), and 30° (○).

purposes is insignificant. The relationship of furfural content to storage temperature is shown in Figure 1 for canned orange juice. Furfural determined over a 16-week period at five temperatures showed that furfural levels increase slowly at 5, 10, and 16°, moderately at 21°, and rapidly at 30°. After 16 weeks the furfural levels per liter of juice were: 533 μg (30°); 131 μg (21°); 66 μg (16°); 32 μg (10°); and 19 μg (5°). Comparative examination of these data shows that juice stored at the highest temperature (30°) for 16 weeks contains approximately 28 times the level of furfural formed in the 5° stored juice. These data also interestingly show that for every 5° rise in temperature there is an approximate doubling of the furfural level. It is apparent that furfural levels correlate in a predictable manner to storage temperatures. It will also be shown in succeeding portions of this paper that furfural correlates closely with flavor differences in canned juice.

The relationship of furfural content to storage temperature for glass-packed orange juice (industrially termed "chilled juice") is shown in Figure 2. The furfural content *vs.* storage time profiles for the five temperatures are similar to those shown for canned juice in Figure 1. After 16 weeks the furfural levels per liter of juice were: 859 μg (30°); 173 μg (21°); 80 μg (16°); 25 μg (10°); and 12 μg (5°). With juice packed in glass, there does not appear to be a simple doubling of furfural content with a 5° rise in storage temperature. The 30° glass-packed juice profile appears to manifest an exponential character which is noticeably different from the essentially linear profile of 30° canned juice shown in Figure 1. The glass-packed juice stored at 30° for 16 weeks contains approximately 72 times more furfural than the 5° stored juice.

Along with the measurement of the furfural content of various temperature stored juices, organoleptic tests were also conducted. In these tests, 5° stored canned juice was organoleptically compared to canned juice stored at the four higher temperatures, and likewise 5° glass-packed juice was compared to other higher temperature stored glass-packed juice. Organoleptic comparison of canned juice to glass-packed juice was not made because the flavor of canned juice was distinctly different from that of glass-packed juice, regardless of the storage temperature. Organoleptic comparison was conducted on all juices until

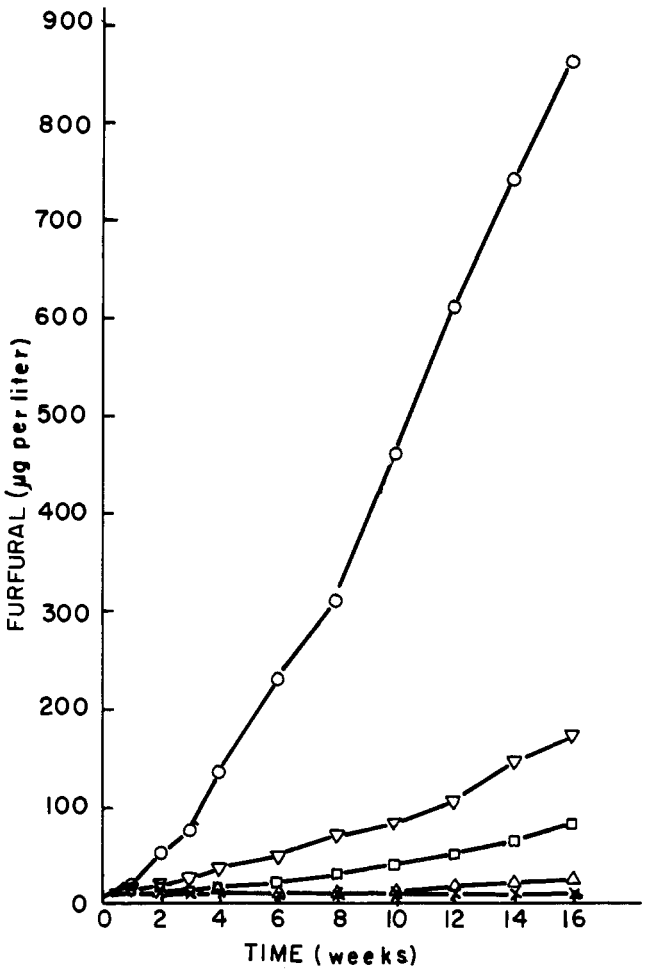

Figure 2. Increase in furfural content in glass-packed orange juice over a 16-week period at 5° (X), 10° (△), 16° (□), 21° (▽), and 30° (○).

the significance of difference was $p < 0.001$, whereupon further taste evaluations were suspended.

Table I shows the relationship of increase in furfural content with time at four storage temperatures and organoleptic evaluation of canned and glass-packed orange juice. With canned juice stored at 10°, no significant taste difference was observed within the 16-week storage period. For the 16° canned juice, a highly significant taste difference ($p < 0.001$) was observed at the 10th week, during which time the furfural level increased to 55 μg/l. Juice stored at 21° showed this $p < 0.001$ value at the 8th week (62 μg/l.), while 30° juice manifested its $p < 0.001$ value at the 2nd week (85 μg/l.). It was evident from these values that the magnitude of flavor difference correlated closely with furfural levels. These data indicated that furfural values within the approximate region 50–70 μg/l. correlated with a difference in flavor in comparison to controls at the significance of difference level of $p < 0.001$. The value reported for 30° juice (85 μg/l.) at the 2nd week was beyond the 50–70 μg region. Dinsmore and Nagy (1972) previously showed that 30° stored juice would develop a significant flavor difference within a 2-week period; therefore the 50–70 μg region was chosen as a more practical region than one extending to 85 μg/l. Although no significance was recorded for 10° canned juice, we predict by linear extrapolation of furfural values to the 50–70 μg/l. region that 10° juice would manifest a significant flavor difference with $p < 0.001$ between 30 and 35 weeks. In like manner, we predict by extrapolation of furfural values for 5° juice that this critical 50–70 μg region would be reached between 72–78 weeks.

The relationship between furfural content and development of flavor differences for glass-packed juice is also shown in Table I. As with canned juice, furfural content correlates closely with changing flavor properties in glass-packed juice. Within the 16-week period, 10° stored juice showed no statistical taste difference to 5° juice. Juice stored at 16° showed a $p < 0.001$ at the 12th week (52 μg/

Table I. Relationship of Furfural Content to Extent of Flavor Changes in Canned and Glass-Packed Orange Juice Stored at 10, 16, 21, and 30°

	10°		16°		21°		30°	
Storage time, weeks	Furfural, ppb	Significance of difference	Furfural, ppb	Significance of difference	Furfural, ppb	Significance of difference	Furfural, ppb	Significance of difference
				Canned orange juice				
1	12[a]	N.S.[b]	15	N.S.	18	N.S.	31	N.S.
2	17	N.S.	27	N.S.	32	N.S.	85	$p < 0.001$
3	21	N.S.	32	N.S.	39	N.S.	120	$p < 0.001$
4	24	N.S.	35	N.S.	47	$p < 0.01$	156	$p < 0.001$
6	24	N.S.	38	N.S.	54	$p < 0.01$	215	$p < 0.001$
8	25	N.S.	42	$p < 0.05$	62	$p < 0.001$	291	$p < 0.001$
10	27	N.S.	51	$p < 0.001$	77	$p < 0.001$	370	$p < 0.001$
12	29	N.S.	55	$p < 0.001$	94	$p < 0.001$	432	$p < 0.001$
14	31	N.S.	61	$p < 0.001$	115	$p < 0.001$	480	$p < 0.001$
16	32	N.S.	66	$p < 0.001$	131	$p < 0.001$	533	$p < 0.001$
				Glass-packed orange juice				
1	9	N.S.	10	N.S.	14	N.S.	18	N.S.
2	9	N.S.	12	N.S.	24	N.S.	55	$p < 0.001$
3	11	N.S.	14	N.S.	27	$p < 0.05$	74	$p < 0.001$
4	11	N.S.	19	N.S.	37	$p < 0.01$	134	$p < 0.001$
6	12	N.S.	23	N.S.	48	$p < 0.01$	239	$p < 0.001$
8	13	N.S.	30	N.S.	64	$p < 0.001$	312	$p < 0.001$
10	13	N.S.	39	$p < 0.01$	81	$p < 0.001$	461	$p < 0.001$
12	15	N.S.	52	$p < 0.001$	105	$p < 0.001$	610	$p < 0.001$
14	19	N.S.	63	$p < 0.001$	145	$p < 0.001$	739	$p < 0.001$
16	25	N.S.	80	$p < 0.001$	173	$p < 0.001$	859	$p < 0.001$

[a] Furfural value represents the mean of three determinations. [b] Not significant at $p < 0.05$.

l.); 21° showed this statistic at the 8th week (64 μg/l.); and 30° juice showed this statistic at the 2nd week (55 μg/l.). As with canned juice, the highly significant statistic $p < 0.001$ was recorded for essentially the same narrow furfural region (50–70 μg/l.) in glass-packed juice. Extrapolation of furfural values for 10 and 5° stored juices to the 50–70 μg region indicates that these juices would evince a flavor difference ($p < 0.001$) at 34–38 weeks for 10° juice and 88–92 weeks for 5° juice.

The origin of furfural in orange juice is unknown. Huelin (1953) believes that furfural originates from the decomposition of ascorbic acid. Huelin conducted an experiment with a 0.25% ascorbic acid in distilled water at 30° for 2 years and found that the two major decomposition products were furfural and carbon dioxide. With lower pH values there was an increase in furfural formation. Studies by Tatum *et al.* (1967, 1969) on nonenzymic browning of orange powder showed that furfural formed during acid-catalyzed hydrolysis of ascorbic acid. It thus appears from the work of Heulin and Tatum *et al.* that ascorbic acid is a likely candidate for being the precursor of furfural in orange juice.

The present study showed the close relationship between furfural levels and temperature storage and the extent of comparative flavor differences. Furfural levels in juice should be regarded strictly as an index of those substances responsible for flavor change. Furfural, *per se*, does not elicit any flavorful properties even when added to a 5° control juice at the 2000 μg/l. level. It is not necessary, however, for the existence of a direct flavor connection with furfural for accepting furfural as a useful and reliable index of changing flavor properties.

In these experiments, formation of furfural apparently parallels closely the formation of components responsible for flavor changes which have, to date, not been identified. There is the possibility that off-flavors may form at a different rate in a different manner than furfural, depending upon the conditions of the juice; *i.e.*, oxygen content, pH, metal ions, °Brix, and other factors. However, a close correlation of furfural content to flavor change has also been demonstrated with canned and glass-packed grapefruit juices (Randall and Nagy, 1972). Although there is the possibility that furfural may not correlate closely with flavor change, the authors have to date not observed this lack of correlation in several studies on canned and glass-packed orange and grapefruit juices.

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Effect of γ Irradiation and Heating on Proteolytic Activity of Meat Samples

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Utilizing a ^{14}C-labeled hemoglobin substrate, the total proteolytic activity of ground beef samples subjected to γ irradiation and/or brief heating (blanching) has been determined. γ irradiation alone, in increasing doses from 2 to 6 Mrad, destroys up to 75% of the proteolytic activity but is less effective than blanching, especially when the latter is carried out at 70°. A combination of irradiation at a dose of 4.5 to 5.2 Mrad plus blanching at 65 or 70° may be expected to destroy at least 95% of the proteolytic activity in beef.

Processing of meats with sterilizing doses of ionizing radiation is a useful new method to make cooked meats shelf stable for long periods of time (JCAE, 1968). However, when applied to raw meats, the shelf stability is limited by the activity of the residual proteolytic enzymes. Early works showed that irradiation alone did not inactivate all the proteolytic enzymes of raw meats. Doty and Wachter (1955) found little reduction in the activity of proteolytic enzymes in fresh beef muscles irradiated with 5×10^5 rep of cobalt-60 γ radiation, whereas irradiation at dosages of 1.6×10^6 rep reduced the apparent proteolytic activity (as measured by liberation of tyrosine from casein substrate) of the beef muscles about 50%. Chiambalero *et al.* (1959) determined a time–temperature relationship for heat inactivation of proteolytic enzymes in beef muscles. He found that proteolytic enzymes are inactivated by heating at 71° for 90 sec and at 77° for 17 sec.

It is obviously important to know the amount of the residual proteolytic activity which remains after various treatments, for it may be assumed that the lower the proteolytic activity, the less deterioration there will be in the stored meat.

In this report the effects of various combinations of irradiation and blanching on proteolytic enzyme activity of

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