accumulator" (Peterson and Butler, 1962). The accumulator plants which can take up several hundred parts per million of Se contain Se in the form of free amino acids, whereas Se in the nonaccumulators is found in the protein. Perhaps a similar difference in the chemical form of Se exists between meat, seafoods, etc., *vs.* vegetables; *i.e.*, perhaps in the former foods Se is rather tightly bound in the protein, whereas in the latter foods Se occurs as relatively unstable free selenium compounds that are easily lost upon heating or cooking.

CONCLUSIONS

The results in this study indicate that the major sources of selenium in the American diet (meats, seafoods, eggs, and cereal products) do not lose appreciable amounts of selenium when cooked by most ordinary methods. On the other hand, some vegetables known to contain relatively high levels of sulfur and selenium (asparagus and mushrooms) do lose significant quantities of selenium as a result of cooking. In spite of these losses, however, it appears that most usual cooking practices will not alter the selenium content of the American diet enough to change the statement that "a diet well-balanced in other nutrients is probably also nutritionally adequate with regard to selenium" (Morris and Levander, 1970). Of course, one must still be on the lookout for possible local variation in the Se content of the diet due to geological factors, especially in light of the recent report of Ullrey *et al.* (1971), who found a significant linear correlation between the Se content of feedstuffs and the tissue concentration of Se in animals from several areas of the U.S.

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Effect of Bruising and Aging on the Alcohol-Insoluble Solids of Red Tart Cherries

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Labeled acetate, citrate, and glucose were administered through the stems of carefully picked cherries. The cherries were then bruised and permitted to metabolize for 2 or 24 hr. The bruised cherries contained 1.55% alcohol-insoluble solids at 24 hr, and their unbruised controls contained 1.29%. The change in the alcohol-insoluble solids in the bruised from that of the control was due to increases in the cellulose and lignin fractions, not in the pectin. Glucose incorporation increased from 167 to 250% in all fractions in the controls between the 2- and 24-hr periods, but showed differences of only from 7 to 110% in the bruised cherries with low or no statistical significance. At 2 hr, acetate and citrate levels were above glucose levels, and were affected much less by bruising and aging.

The bruising and aging of Montmorency cherries have been shown to affect the firmness, water-holding capacity, and other characteristics in complex ways, especially in combination with other treatments (Whittenberger, 1952; Hills *et al.*, 1963; Buch *et al.*, 1961). Previous studies, which helped to elucidate some of the biochemical changes underlying these effects, showed that bruising altered respiration and conversion of carbon from acetate and citrate to carbon dioxide (Pollack *et al.*, 1958a,b, 1965) and induced callose formation (Dekazos and Worley, 1967). The studies presented here show further effects of bruising on the overall composition and substrate incorporation into the structural material of the cherries.

EXPERIMENTAL

On three alternate days during the harvest season cherries were harvested, as described previously by Pollack *et al.* (1958b), by cutting the stems gently while supporting the cherries carefully with a cotton pad. The harvested cherries were treated in groups of ten, and the cherries in each group were subsequently combined for homogenization and extraction.

To administer the substrates, 10-μl amounts of the substrates were placed in micro test tubes (about 3 mm × 10 mm). The stems of the cherries were then inserted into the solutions

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Table I. The Effect of Bruising and Aging on Content of Alcohol-Insoluble Solids and Its Components in Red Tart Cherries

Incubation time	Percentage (g of each component per 100 g wet cherry wt)				Percent change 24 hr *vs.* 2 hr	
	2 hr		24 hr			
	Normal	Bruised	Normal	Bruised	Normal	Bruised
AIS[a]	1.22 ± 0.09[b]	1.33 ± 0.07	1.29 ± 0.04	1.55 ± 0.09	6 <0.05[c]	17 <0.001
Pectin	0.59 0.08	0.53 0.03	0.64 0.05	0.52 0.03	8 <0.2	−2 <0.5
Lignin	0.34 0.07	0.41 0.05	0.32 0.07	0.54 0.04	−6 <0.8	32 <0.001
Cellulose	0.29 0.03	0.38 0.03	0.34 0.03	0.49 0.05	17 <0.02	29 <0.001

[a] Alcohol-insoluble solids. [b] Standard deviation. [c] Probability of difference being due to statistical variability according to standard "t" test.

Table II. Incorporation of the Different Substrates into the Cherry Fractions

Incubation time	Percent of applied radioactivity recovered in the fraction				Percent change 24 hr *vs.* 2 hr	
	2 hr		24 hr			
	Normal	Bruised	Normal	Bruised	Normal	Bruised
Glucose-U-^{14}C						
AIS[a]	0.47 ± 0.09[b]	0.35 ± 0.10	1.29 ± 0.35	0.53 ± 0.11	174 <0.05[c]	51 ...[d]
Pectin	0.18 0.02	0.14 0.04	0.48 0.12	0.18 0.13	167 <0.02	29 ...
Lignin	0.17 0.04	0.12 0.04	0.48 0.03	0.21 0.04	182 <0.001	75 ...
Cellulose	0.06 0.01	0.06 0.02	0.21 0.07	0.13 0.05	250 <0.05	117 ...
Citrate-1,5-^{14}C						
AIS	0.74 ± 0.07	0.78 ± 0.20	0.96 ± 0.06	0.79 ± 0.24	30 <0.001	1 ...
Pectin	0.19 0.03	0.18 0.03	0.24 0.04	0.19 0.04	26 ...	6 ...
Lignin	0.54 0.04	0.48 0.17	0.58 0.18	0.52 0.10	7 ...	8 ...
Cellulose	0.09 0.03	0.15 0.04	0.19 0.07	0.22 0.07	110 <0.02	47 ...
Acetate-1-^{14}C						
AIS	0.98 ± 0.46	1.04 ± 0.25	1.20 ± 0.18	1.32 ± 0.24	22 ...	27 ...
Pectin	0.35 0.11	0.38 0.03	0.42 0.04	0.44 0.68	20 ...	16 ...
Lignin	0.45 0.07	0.38 0.11	0.46 0.13	0.52 0.12	2 ...	37 ...
Cellulose	0.14 0.05	0.16 0.02	0.19 0.07	0.25 0.01	36 ...	56 <0.001

[a] Alcohol-insoluble solids. [b] Standard deviation. [c] Probability of difference being due to statistical variability according to standard "t" test. [d] Probability less than 90% confidence level.

and left until the solution was drawn up into the stem and cherries. Two successive 10-μl portions of water were then added to each test tube to be drawn up and thus wash the labeled solution completely into the cherries.

With each day's harvest, separate sets of normal and bruised cherries, containing the labeled substrates, were simultaneously incubated. Analyses of each final batch of cherries were carried out in duplicate. The 2-hr interval was chosen to obtain early indications of any effects, and the 24-hr time period allowed effects to develop without allowing extensive deterioration to occur.

Some cherries were then bruised by rolling them between two glass plates until they became flaccid, taking care to avoid breaking the skin. The bruised fruits and their unbruised controls were left to stand at 30 ± 2°C for 2 or 24 hr. Following incubation the stems and seeds were removed and the cherries extracted with hot 80% (v/v) ethanol, including the cherry weights as part of the water, according to the procedure of Jermyn (1955). The alcohol-insoluble solids were fractionated by removing pectins with hot water, removing lignin with sodium chlorite and acetic acid, and leaving final cellulose residue (Jermyn, 1955).

The radioactive compounds administered were sodium acetate-1-^{14}C, 3.5 × 10^6 decompositions per minute (DPM)/cherry; citric acid-1,5-^{14}C, 2.4 × 10^6 DPM per cherry; and glucose-U-^{14}C, 110 × 10^6 DPM per cherry. They were purchased from Tracerlab, Inc. Samples were counted in a Packard Tri-Carb scintillation counter. Samples of the total alcohol-insoluble solids and of the cellulose were ground, suspended in a scintillation medium containing Cab-O-Sil, sonicated for 2 min, and then counted.

RESULTS AND DISCUSSION

The increase in content of total alcohol-insoluble solids in the bruised cherries, small at 2 hr and greater at 24 hr, is shown in Table I, and can be seen to be due to increases in the lignin and cellulose components only.

As can be seen in Table II, glucose is the only substrate used here whose uptake into all fractions tested was significantly affected by bruising and aging. Its uptake increased greatly on 24-hr incubation in the controls, but the bruised cherries showed much less and a less statistically significant increase. The lower 2-hr uptake of glucose than of acetate and citrate is contrary to what would be expected from the apparent need to convert acetate and citrate to glucose phosphate before their incorporation into the polysaccharides. No explanation for this phenomenon is presently available.

These studies thus confirm and extend earlier conclusions (Buch *et al.*, 1961) that bruising cherries does induce changes in their polysaccharide content. The differences shown here in the direction of change of pectin from that of lignin and cellulose may help explain some of the variability in the effect of bruising on water-holding capacity of the cherries. Callose (Dekazos and Worley, 1967) was not specifically sought in this study. It would be expected to comprise part of the cellulose fraction in the fractionation procedure and may contribute to the observed increase in cellulose content.

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Structural Identification of the Methoxymethylpyrazine Isomers

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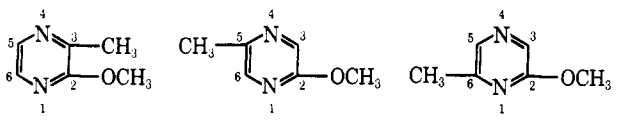
The methoxymethylpyrazine isomers were synthesized and isolated. They were characterized by gas chromatography, infrared, Raman, and nuclear magnetic resonance techniques. The 2-methoxy-3-methyl- and the 2-methoxy-6-methylpyrazine were obtained according to published procedures. A new route for the synthesis of 2-substituted 5-methoxypyrazines was used in the preparation of 2-methoxy-5-methylpyrazine. The authors believe this is the first time that this compound has been isolated and characterized.

The methoxymethylpyrazines are of considerable interest to workers in the area of flavor because of their organoleptic properties. This is borne out in a Firmenich patent (Firmenich *et al.*, 1967) which deals with the three isomers as well as with related compounds. The methoxymethylpyrazines exhibit strong and characteristic "baked"



2-methoxy-3-methylpyrazine | 2-methoxy-5-methylpyrazine | 2-methoxy-6-methylpyrazine

flavor notes and have potential application as additives to a variety of baked or toasted goods. The higher alkyl homologs of the methoxymethylpyrazines are very different in flavor and odor intensity from their parent compounds; this has been demonstrated with 2-methoxy-3-methylpyrazine and its homologs (Murray *et al.*, 1970; Seifert *et al.*, 1970). We can assume that subtle but important flavor differences exist also among the pure methoxymethylpyrazine isomers themselves. These differences may, at least in part, be deciding factors in achieving natural or true flavor characteristics in specific flavor compositions. Therefore, it was of interest to us to prepare the three methoxymethylpyrazines in pure form and to assign to each isomer the correct structure.

APPARATUS

Isolation and purification of compounds was accomplished using an F&M research chromatograph (No. 5750) employing the thermal conductivity detector. A $^1/_4$-in., 10-ft long stainless steel column packed with 10% Carbowax 20M on Chromosorb W, temperature programmed from 100 to 200°C at 8°C/min, was used to isolate the three methoxymethylpyrazine isomers from the various reaction mixtures. The methylpyrazine *N*-oxide isomers, used as starting materials in the preparation of the methoxymethylpyrazines, were isolated using a $^1/_4$-in, 10-ft long stainless steel column packed with 10% SF96 on Chromosorb W; the column temperature was maintained at 140°C.

Infrared spectra were recorded with a Perkin-Elmer model 621 grating infrared spectrometer. Samples were run as neat liquids between cesium bromide plates. Nuclear magnetic resonance spectra were recorded with a Varian HA-100 spectrometer using the capillary microtechnique as described by Haynes and Sazavsky (1970). A Cary 81 Raman spectrometer equipped with an argon ion laser source was used for recording Raman spectra on neat samples contained in sealed capillary tubes.

REAGENTS

2,3-, 2,5-, and 2,6-Dimethylpyrazines. These compounds were commercially obtained and were purified by gas chromatography.

2-Methylpyrazine 1-Oxide and 2-Methylpyrazine 4-Oxide. A mixture of the two methylpyrazine oxides was prepared as described by Koelsch and Gumprecht (1958). Several milligrams of each isomer were isolated by gas chromatography using the column conditions specified above. The melting points of the isolates were 90°C (2-methylpyrazine 1-oxide) and 64°C (2-methylpyrazine 4-oxide). Structural assignments of these isomers are based on the discussions of Gumprecht *et al.* (1964).

PROCEDURES

2-Methoxy-3-methyl- and 2-Methoxy-6-methylpyrazine. Firmenich *et al.* (1967) report the preparation of the three methoxymethylpyrazine isomers by two methods. In the first method the methoxymethylpyrazines were obtained by

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