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SUMMARY OF DATA ON RAISIN JUICE CONCENTRATE

Abstract. Raisin juice concentrate is used as flavoring. It has not been evaluated by FEMA for GRAS status.

Two mouse skin-painting bioassays determined that raisin juice concentrate does not contribute to the carcinogenic potential of cigarette smoke. In inhalation studies, raisin juice concentrate flavored cigarettes caused an increase in the size of mouse lung epithelial cells, and an increase in the number of mitotic figures in rat tracheas. However, two other inhalation studies on rat respiratory tracts and mouse bone marrow cells showed no significant effect of exposure to raisin juice flavored versus unflavored cigarettes.

Raisin juice concentrate did not induce hepatic microsomal activity in rats or mice when administered orally, nor did it induce immunosuppressive activity in mice by that route. Raisin juice concentrate also had no effect on the cardiovascular and respiratory systems of three anesthetized young adult female beagle dogs. The LD_{50} for rats is greater than 5000 mg/kg.

Raisin juice concentrate was not mutagenic in an Ames' assay up to 10000 pg/plate, nor genotoxic in a mouse lymphoma cell forward mutation assay. However, extracts of the compound were mutagenic to TA98; these results were attributed to the presence of quercetin and kaemferol in the extract. The extracts were also clastogenic in CHO cell cultures, but this may be due to the extremely large amounts used.

There is no information available concerning the human health effects, chronic toxicity or reproductive toxicity of raisin juice concentrate. The physical and chemical compositions of raisins and grapes are discussed in Appendices A and B respectively.

I. Background. Raisin juice concentrate (CAS No. 68915-86-6) is made from raisins (dried grapes) derived from varieties of the species Vitis vinifera (family Vitaceae).

This species is native to Western Asia and is grown extensively in areas (Mediterranean, California, Australia, and South Africa) where hot and dry summers predominate (Halsey et al., 1968; Considine and Considine, 1983a). More raisins are produced worldwide than any other dried fruit (Bolin and Petrucci, 1985). Whole raisins are eaten without preparation and are used extensively for home and commercial cooking and baking.

Ripe grapes contain approximately 60-85 percent water (Amerine and Joslyn, 1973a; Amerine et al., 1972a), while raisins ideally contain 12.0-14.0 percent water (Clingeleffer, 1984; Bolin and Petrucci, 1985). In order to achieve this level of uniform dryness during ground drying, the grapes usually are spread out at a depth of 25 mm (3-4 berries deep). Approximately four pounds of fresh grapes produce one pound of raisins (Heath, 1981). About 85 percent of the grapes dried for raisins and raisin juice are of the Thompson seedless variety, also known as the Sultana variety (Heath, 1981). The Muscat of Alexandria seeded variety and the Black Corinth seedless variety also are used in raisin production (Heath, 1981; Considine and Considine, 1983a).

Various methods are used to process raisins from ripe grapes (Halsey et al., 1968; Considine and Considine, 1983a). Grapes can be either sun-dried, or artificially dried in dehydrating tunnels, where they are exposed to warm dry air for about 18 hours. Dried grape aroma differs from fresh

grape aroma and depends on the grape's drying conditions (Ramshaw and Hardy, 1969). Prior to drying, the grapes may be dipped in a hot, caustic solution (2 percent dipping oil composed of c14-c18 fatty acid esters in a 2.5 percent aqueous solution of potassium carbonate) to remove the waxy coating (bloom) and crack the skin (Ramshaw and Hardy, 1969). Grapes dried on the vine may be sprayed with a fatty acid emulsion of ethyl oleate mixture and potassium carbonate (Bolin and Petrucci, 1985). These procedures hasten drying by altering the arrangement of surface wax platelets, making the skins more permeable (Bolin and Stafford, 1980). However, fatty acid esters are persistent and often leave off-flavors. Guadagni et al. (1975) demonstrated that methyl oleate is desirable for raisin drying because it has a taste threshold that is 16 times higher than does ethyl oleate. Sulfur dioxide treatment is employed prior to drying for its antimicrobial properties and ability to inhibit oxidative discoloration (Amerine and Joslyn, 1973b; Considine and Considine, 1983a). Potassium sorbate also can be used for antimicrobial protection (Considine and Considine, 1983a). During grape maturation there is an increase in the proportion of nitrogen (amino acids, ammonia, and amines) which cannot be utilized by yeast. Due to this phenomenon, some natural antimicrobial activity develops as grapes turn to raisins. Overripe grapes therefore often ferment at a slower rate than fresh grapes.

II. Use in Tobacco.

A. Function. Raisin juice concentrate is used as a flavorant in cigarette tobacco. As tobacco ingredients, fruit juices such as raisin juice add sweetness to the flavor and smooth the smoke (unpublished observations). Their characteristic taste and aroma are reportedly due to the synergistic action of aromatic components (alcohols, carbonyls, volatile acids such as formic and acetic, esters, lactones, and phenols), fruit sugars and non-volatile fruit acids such as malic, tartaric and citric (Heath, 1981).

B. Use Level. The Industry maximum use level for raisin juice concentrate is 2.26%. Raisin juice concentrate has not been evaluated for GRAS status by FEMA and does not appear in the Food Chemicals Codex. Great Britain's "Hunter" Report on Smoking and Health (Froggatt, 1988) lists fresh or dried raisin fruit or extract as permissible in cigarette tobacco at 4.0 percent levels.

III. Chemistry & Pyrolysis

A. Chemistry. The physical and chemical composition of raisins (processed product) and grapes (raw materials) are described in Appendices A and B, respectively.

B. Pyrolysis Studies. There is currently insufficient experimental data on the pyrolysis of raisin juice to establish a definitive characterization of this complex mixture.

IV. Toxicology Studies.

A. Metabolism. A study to evaluate the potential of raisin juice concentrate to induce hepatic microsomal enzyme activity was performed in CD-1 mice and Sprague-Dawley rats (unpublished studies). Groups of five animals/sex/species were administered oral doses of 2500 mg/kg raisin juice concentrate in 1 percent methyl-cellulose for four consecutive days. Phenobarbital was employed as a positive control. The effect of this pretreatment on hexobarbital sleep times was assessed for both sexes of mice, and hepatic microsomal enzyme suspensions were prepared from the rats. Raisin juice concentrate had no effect on hexobarbital sleep nor on liver-to-body weight ratios in rats of either sex. p-Nitroanisole 0-demethylase activity did increase in dosed male rats, and aniline hydroxylase activity significantly decreased in female rats. However, changes were not considered to be biologically significant. Based on the results, raisin juice concentrate did not have any meaningful effect on several measures of xenobiotic metabolism in two rodent species.

B. Acute Human Toxicity. There is no information available on the acute human toxicity of raisin juice concentrate.

C. Chronic Animal Studies. There are no available chronic animal studies concerning raisin juice concentrate.

D. Acute & Subchronic Animal Studies. Nose-only inhalation studies have been performed to compare the toxicity of smoke from reference cigarettes and those containing 57,000 ppm raisin concentrate by weight (unpublished studies). These studies exposed B6C3F1 mice and F344 rats to cigarette smoke for 30 seconds, followed by a 30-second exposure to fresh air. This cycle was repeated eight times to constitute one exposure (one cigarette). The animals were rested for eight minutes and then the exposure cycle was repeated. Generally, a total of nine cycles (72 individual 30-second exposures) were completed during each day of the study. The smoke concentration for all of the studies was 10 percent (approximately 4 pg particulate matter/ml of air).

Acute exposure of mice and rats to smoke from reference and raisin concentrate-flavored cigarettes did not result in substantial differences in toxicity (unpublished study). Subchronic inhalation studies (five days/week for six weeks) were conducted in mice and rats to assess a variety of toxicologic parameters, i.e.: survival, body weight, clinical pathology, lung weights, mitotic activity of lung tissue, and respiratory tract histopathology (unpublished studies). The only significant finding in mice was an increased incidence of diffuse hypertrophy of lung epithelial cells in those animals exposed to smoke from cigarettes containing raisin concentrate (1/6 in reference group, 5/6 in test group).

The studies using rats indicated that the number of mitotic figures in the trachea was increased in those animals exposed to smoke from cigarettes containing raisin concentrate. However, histopathological examination of the respiratory tissues did not indicate any significant difference between the two cigarette types. The increased mitotic activity in the rats' tracheas suggests a higher level of cell proliferation. Microscopic quantitation of mitotic indices in respiratory tract tissue sections may be difficult, however, due to the significant influence of the angles and planes of the sections on the apparent number of mitoses. Therefore, the biological significance of the increased mitotic activity is questionable.

A further evaluation of the potential of the raisin juice concentrate flavored cigarette to affect the respiratory tract was performed. In another subchronic inhalation study conducted in F344 rats, male rats received six 30-second exposures nine times/day, while females received seven 30-second exposures nine times/day (unpublished studies). The animals were exposed to the smoke condensates five days/week for thirteen weeks. Parameters examined included: survival, body weight, clinical pathology, urinalysis, major organ weights, lung lavage fluid cytology and biochemistry, and histopathological evaluation of major organs and the respiratory tract. The results indicated no significant biological differences between the animals exposed to smoke

from the flavored cigarettes and those exposed to the reference cigarettes.

Another smoke inhalation study was performed to assess the differences in the ability of raisin concentrate flavored cigarettes to induce sister chromatid exchanges (SCE) in bone marrow cells in mice. For these studies, the smoke exposure was increased to 18 exposures/day for ten consecutive days (seven 30-second puffs/exposure). No differences in SCE rates were observed between those mice exposed to the smoke of the unflavored and flavored cigarettes.

The acute toxicity of raisin juice concentrate was assessed by the oral administration in a 0.9 percent saline vehicle to male and female Sprague-Dawley rats (unpublished study). Five animals of each sex received a dose of 5000 mg/kg raisin juice concentrate following an overnight fast. The animals were maintained and observed for a 14-day period following dosing. No mortality was observed in either sex at this dose level. Based upon these results, the LD_{50} was estimated to be greater than 5000 mg/kg.

A study to assess the potential of raisin juice concentrate to affect the function of the cardiovascular and respiratory systems was conducted in three anesthetized young adult female beagle dogs (unpublished study). Ascending doses of 0.4, 0.8, and 2.0 mg/kg were delivered in a 0.9 percent saline vehicle by slow jugular infusion as a battery of parameters were recorded including: aortic pressure, lead II

of the ECG, tidal volume, and respiratory rate. A variety of parameters of cardiovascular and respiratory function were calculated from the recorded values including: heart rate, stroke volume and work, cardiac index, systemic and segmental vascular resistance, and minute volume. No physiological impairment of the cardiovascular or respiratory systems was observed after administration of raisin juice concentrate.

E. Skin Painting Studies. While a relationship between mouse skin tumorigenesis and human illness or fatality has not been established, this assay technique has been used in the evaluation of the biological effects of cigarette smoke condensates for some time. Mouse skin-painting bioassays were conducted to assess the tumor-promoting potential of smoke condensate from tobacco cigarettes to which raisin concentrate had been added at 57,000 ppm in a compound flavor (unpublished studies). Groups of 60 female ICR CD-1 mice received subthreshold, initiating doses of 7,12-dimethylbenz-(a)anthracene prior to four applications of 30 or 45 mg of the cigarette smoke condensates (CSC) per week for 26 weeks in one experiment and 20 or 35 mg CSC for 40 weeks in another experiment. The condensates of the raisin juice concentrate flavored cigarette and an unflavored reference cigarette were comparable in tumor-promoting activity, with no significant differences in grossly observed or histologically confirmed tumor incidence at either dose. The results of these assays provide evidence that neither raisin juice concentrate nor its

pyrolysis/combustion products contribute significantly to the tumorigenic activity of cigarette smoke condensates.

F. Genotoxicity & Mutagenicity. The potential mutagenicity of raisin juice concentrate was assessed in Ames' Salmonella strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100, both in the absence and presence of an Aroclor-induced rat liver S9 preparation (to simulate mammalian metabolism) (unpublished study). Eight levels of raisin juice concentrate, ranging from 1.0 to 10,000.00 pg/plate, were assayed in triplicate. No mutagenic response was observed under any of the conditions in this assay, providing evidence that neither raisin juice concentrate nor its metabolic products are genotoxic.

Stolz et al. (1984) found that extracts prepared from dried raisins were mutagenic to Salmonella strain TA98 in the presence of a mammalian liver S9 mix. The authors attributed the mutagenic activity of the extract to the flavonoids quercetin and kaemferol, which are natural constituents of raisins. The relevance of this study to any potential health effect(s) is unclear, however, because these findings were obtained from extracts of raisins in a cell culture system. In addition, flavonoids are widely consumed (grams/day), ubiquitous natural constituents which occur in fruits, vegetables, herbs and spices, legumes, cereal grains, tea, and cocoa and traditionally have been regarded as safe for consumption (Horowitz, 1981; Hertog et al., 1992; Bilyk

and Sapers, 1985; Brown, 1980; Herrmann, 1976; Spanos and Wrolstad, 1992). There are numerous reports that flavonoids including quercetin and kaemferol are also natural constituents of the tobacco leaf (Dawson and Wada, 1957; Snook and Chortyk, 1982; Herrmann, 1964; Court, 1986; Andersen et al., 1969) but that they pyrolyze to simpler phenolic compounds during smoking conditions (Zane and Wender, 1963; Kallianos et al., 1967). To add to the confusion it is still generally unclear whether flavonoids are beneficial or harmful to humans (Hertog et al., 1992) due to conflicting reports that they have both mutagenic/carcinogenic (Bjeldanes and Chang, 1977; Mazaki et al., 1982; Pamukcu et al., 1980; Vrijsen et al., 1990; Brown, 1980; Dunnick and Hailey, 1992) and antimutagenic/anticarcinogenic (Das et al., 1987; Buening et al., 1981; Wargovich et al., 1985, Deschner et al., 1991; Kato et al., 1983; Huang et al., 1983) properties. It is interesting that the flavonoid (+)-Catechin inhibits the bioactivation of the tobacco-specific N-nitrosamine-4-(methylnitrosamino)-1-3-pyridyl)-1-butanone (NNK) and NNK-induced clastogenesis of hepatocytes in cell culture (Liu and Castonguay, 1991). It is clear that issues involving the biological properties of flavonoids are very complicated and must be considered in the context of the complex mixture to which they are a component.

Raisin juice concentrate was evaluated for genotoxic potential in an L5178Y TK+/- mouse lymphoma cell forward

mutation assay, both in the absence and presence of an Aroclor-induced rat liver S9 metabolic preparation. Levels of raisin juice concentrate ranging from 250 to 5000 nl/ml were assayed without activation, and levels of 62.5 to 5000 nl/ml were assayed in the presence of S9. No significant increases in the mutant frequency at the TK locus were observed, providing evidence that neither raisin juice concentrate nor its metabolites are mutagenic in mammalian cells.

Stich et al. (1981) assayed aqueous extracts of dried fruits for clastogenic potential in CHO cell cultures, both in the absence and presence of S9 mix. All of the dried fruit extracts tested, including raisin extracts, were identified as having "strong genotoxic activity" in this in vitro assay. Addition of the S9 mix to simulate mammalian metabolism reduced the clastogenic effects in all instances. The authors did not discuss the possible contribution of osmotic shifts in the cell culture medium to the occurrence of irrelevant clastogenesis under the conditions of the assay. The authors' conclusion that raisin extracts are potently clastogenic is based on extraordinarily high extract levels (up to 50 percent extract in culture medium, v/v, or 60 mg lyophilized extract/ml medium), and should be viewed with caution.

G. Reproductive Toxicity & Teratology. There are no available studies concerning the reproductive toxicity of raisin juice concentrate.

H. Immunotoxicity. Another study addressed the immunotoxicity of raisin juice concentrate (unpublished study). Raisin juice concentrate was orally administered to eight male mice at 2500 mg/kg for 11 consecutive days. Control groups received a 1 percent methylcellulose vehicle alone or with the immunotoxin 6-mercaptopurine. All animals received an antigen in a single 0.3 ml IP dose (25 percent suspension of sheep red blood cells) on the third day of dosing. The animals were sacrificed on the day after dosing was completed, a hemagglutination assay was performed, and spleen, thymus, and adrenal weights were recorded. No suppression of antibody production was observed in animals treated with raisin juice concentrate, nor were spleen/body weight, adrenal/body weight or thymus/body weight ratios affected. It was decided that raisin juice concentrate did not induce immunosuppressive activity in mice under the conditions of this assay.

Appendix A

Physical & Chemical Composition of Raisins

Raisins are considered a high-energy, nutritious food, and contain many minerals and vitamins (Bolin and Petrucci, 1985). Raisin odor and flavor are characterized by a burnt caramelized sensation (Amerine and Roessler, 1976). The important differences among the constituents of raisins and grapes depend on the fruit's drying conditions and resulting moisture content (Ramshaw and Hardy, 1969; Buttery et al., 1981).

Ramshaw and Hardy (1969) compared the volatile constituents of fresh sultana grapes to dried sultana grapes (raisins) using a combined capillary gas chromatography-mass spectrometry method. Some sultana grapes were dried for two weeks after being immersed in a dipping solution (2 percent dipping oil of C14-C18 fatty acids in 2.5 percent aqueous potassium carbonate), while the other sultana grapes were dried for five weeks by sunlight. The grape constituents were extracted using steam distillation followed by trichlorofluoromethane (Freon 11) extraction. The differences in the constituent profiles were attributed to the moisture content (grape juice at 70-85 percent H_2O, dipped raisins at 13.5 percent H_2O, and sun-dried raisins at 10 percent H_2O), the temperature, and other processing variables. Hexan-1-ol, hex-3-en-1-ol, hex-2-enal, and an unidentified compound

composed most of the volatile components of the fresh sultana grape extract. By contrast, more than 40 major components were identified in the two raisin extracts. The major components of dipped sultanas were the fresh fruit volatiles hexan-1-ol, hex-3-en-1-lol, n-alkan-2-ones, γ-butyrolactone, hexanal, hex-2-enal, and diacetyl. The major volatile constituents of undipped sultanas were furfural, methyl furfural, 2-hydroxybutan-3-one, and diacetyl. These compounds and other furan derivatives are associated with the caramelization of sugars and non-enzymatic Maillard-type reactions in food. It is possible that enzymatic browning involving polyphenol oxidase may have contributed to the constituents of sun-dried raisins. Dipped sultana raisins contained several n-alkan-2-ones which were found in fresh grape juice as well, but not in the undipped sultana raisins. 2-Phenyl ethanol, a major product of grape juice fermentation, was also detected in fresh grapes and undipped sultana raisins, but not in dipped sultana raisins.

Buttery et al. (1981) evaluated sultana raisins (dried to 14 percent moisture) for volatile oils and described several constituents. Their methods involved vacuum steam distillation, hexane extraction, and capillary GLC-mass spectrometric quantification. The major components of sultana raisins included aliphatic acids (octanoic, 4.0-8.4 percent; nonanoic, 3.0-5.5 percent), an anhydride (2-hexyl-3-methylmaleic anhydride, 5.0 percent), aldehydes

(phenylacetaldehyde, 4.0 percent; (E)-2-heptenal, 2.1 percent; (E)-2-octenal, 2.7 percent; (E,E)-2,4-decadienal, 2.0 percent), furans (2-pentylfuran, 3.4 percent; 5-methyfurfural, 2.2 percent), and an alcohol (1-octen-3-ol, 2.6 percent). The major components, except for phenylacetaldehyde and 2-hexyl-3-methylmaleic anhydride, are thought to be derived from lipid oxidation.

Bolin and Petrucci (1985) recently characterized the mean amino acid composition (moisture-free basis) of sultana raisins which were processed by a variety of methods (sun-dried, hot-air-dried, dried on the vine after treatment with a fatty acid ester emulsion of an ethyl oleate mixture and potassium carbonate). Grapes were dried to a 12-14 percent moisture level. Arginine was present in the fruit at the greatest concentration (0.739 g/100g; 25 percent of all amino acids), followed by proline (0.400 g/100g; 14 percent of all amino acids) and glutamic acid (0.249 g/100g; 9 percent of all amino acids). These amino acids also predominated in the grape musts (Amerine et al., 1972a) with the highest arginine levels reported in raisins (Bolin and Petrucci, 1985).

Appendix B

Physical & Chemical Composition of Grapes

At harvest, the relative weight and water content of the parts of the grape cluster are as follows:

Part	% Weight	% Water
Stems	2-6	60-80
Berry	95-97	
Skin	5-12	70-80
Pulp	85-87	60-85
Seeds	0-5	30-40

The berry is composed of a fleshy pericarp (pulp) surrounded by skin, and may or may not contain seeds. Each of these regions contributes different chemical constituents to the must or juice of crushed stemmed grapes. The skin is covered with protective surface waxes, which form the layer called the "bloom". Most of the aroma, coloring, and flavoring constituents are in the outer layers of the grape, primarily in the skin. The chemical composition within the grape differs due to the unequal ripening of the pulp; a grape ripens from the exterior to the interior. The berry's change in turgidity is very important during the ripening process (Amerine et al., 1972a). Unripe berries are hard, and difficult to crush. As the fruit ripens, its turgidity increases, which facilitates crushing. If overripened, the

fruit shrivels and loses turgidity, again making it difficult to crush. Bolin's studies (1976) found that heat-treated raisins remain softer than untreated raisins.

The term "must" refers to the free-run juice derived from crushed, unfermented, stemmed grapes (Amerine and Joslyn, 1973a). On a relative weight basis, the must is composed of 2-6 percent stems, 0-5 percent seeds, and 80-90 percent juice. Due to the diverse character and large quantity of musts, their constituents are usually described according to their chemical class and anatomical derivation from within the grape cluster. The following is a broad overview of these constituents (Amerine and Joslyn, 1973a; Amerine et al., 1972a; Amerine, 1984, Amerine and Ough, 1974):

1. Carbohydrates: The two most important carbohydrates in grapes are d-glucose and d-fructose. At least 12-27 percent of the must weight at harvest consists of these sugars, and this percentage continues to increase during ripening. Unripe fruit initially contains slightly less fructose than glucose. However, during ripening the increase in fructose is greater than that for glucose, resulting in higher fructose levels in the ripe fruit. Since fructose is considerably sweeter than glucose, the resulting must is usually sweeter than the unripe fruit. Other carbohydrates occurring at much lower levels include the polysaccharide pectin (0.02-0.6 percent), the disaccharide sucrose

(0.019-0.18 percent), pentoses (0.08-0.2 percent; arabinose, rhamnose, xylose), and inositol (0.02-0.08 percent).

2. Acids: Important organic acids present in grape juice are l-citric, l-malic, and d-tartaric acid. Grape musts contain approximately 0.3-1.5 percent organic acid, 0.2-1.0 percent tartaric acid, 0.1-0.8 percent malic acid, and 0.01-0.05 percent citric acid. Tartaric and malic acid account for over 90 percent of the total acid constituents in grapes. During ripening, the total titratable acidity of the grapes gradually decreases while the pH slowly and steadily increases from 2.8-3.1 or higher. In addition, the total levels of tartrate and malate decrease during ripening; the malate decreases more significantly. As with the sugars, these changes occur in the pulp, moving from the skin toward the seeds. In contrast to the malate and tartrate changes in grape pulp, the acid components increase in the stems and skin during maturation.

3. Nitrogenous Compounds. The nitrogenous compounds found in grape must consist primarily of proteins, amino acids, ammonia, peptides, and amines. The total nitrogen content ranges from 100-1100 mg/L, the protein nitrogen content ranges from 18-40 mg/L, and ammonia nitrogen varies from 5-150 mg/L. Total organic nitrogen in must, expressed as protein, ranges 0.01-0.20 percent. Thus the total nitrogen content is high, while the protein content is low. During maturation, the organic nitrogen (amino acids and proteins)

steadily increases in the pulp, while ammonia levels decrease. The nitrogen profile, primarily organic nitrogen, also changes during ripening. As a result, there are significant increases in proline, serine, and threonine, and decreases in arginine, ammonia, and amine nitrogen. This reaction represents an increase in the nitrogen forms, which are less easily utilized by yeasts, and possibly explains why overripe grapes ferment more slowly.

4. Phenolics. Phenolic substances are very important to the taste, odor, and color of wines (Singleton and Noble, 1976). A large number of these constituents also occur in grape musts (Amerine and Joslyn, 1973a). Anthocyanin, flavonoid, and tannin pigments are the primary phenolic pigments of grape must. Although these phenolic pigments are chemically distinct, they share many structural similarities. A discussion of these similarities is beyond the scope of this review. The color of these pigments, which may exist as glycosides, is modified by organic acids, co-pigments, pH, metals, and sulfur dioxide. Thus, the overall color of grapes depends on a pigment complex of variable composition. Flavonoids and other sizeable phenolics have no significant odor in the pure state, nor do highly polar phenol derivatives such as glucosides and gallic acid (Singleton and Noble, 1986). The anthocyanins have a mild, undistinctive flavor. By contrast, the tannins, which compose approximately 0.01-0.2 percent of grape must, have an astringent taste accompanied by

contracting (puckering) and drying sensations. This characteristic is based on the ability of the tannins to precipitate proteins.

The average total extractable phenol concentration (mg Gallic Acid Equivalent; GAE) in ripe grape berries is 5,500 mg GAE/kg in red grapes and 4,000 mg GAE/kg in white grapes. The typical distribution of total phenols in red grapes is 33.3 percent in skins, 0.7 percent in pressed flesh, 3.4 percent in juice, and 62.6 percent in seeds. These values for white grapes are 23.2, 0.9, 4.5, and 71.4 percent, respectively.

Many grape must phenolics are classified as polyphenolics. Polyphenols represent one of the principal oxidation-reduction systems of musts (Amerine and Joslyn, 1973a). Oxidation of these pigments produces brownish-colored compounds. The auto-oxidation of phenols is accelerated in the presence of ions such as Cu, Fe, Mn, Ni, and Co. Enzymatic browning reactions also are mediated by polyphenol oxidase (PPO), a copper-containing enzyme (Oszmanski and Lee, 1990). Ascorbic acid and peptides from honey inhibit the enzymatic browning resulting from this enzyme. Limited oxidative browning in wines also is attributed to Maillard-type amino-carbonyl reactions (Hashiba et al., 1981).

Immature grapes contain chlorophyll, which gradually diminishes during ripening (Amerine and Joslyn, 1973a; Amerine et al., 1972a). Polyhydroxylated flavones or flavonols, including quercitin, quercetin, and kaemferol, are present in

grapes and grape leaves. Flavone pigments increase in white grapes during ripening. The source of the natural yellow pigment in the plants is substituted flavones. Anthocyanin pigments are amphoteric; acids increase the red tint, and alkalies increase the blue tint. These pigments increase in red grapes during ripening. Tannins occur chiefly in the skins, stems, and seeds of grapes, but decrease by over 50 percent in the fruit during ripening. Although very little tannin is found in the free-run juice from mature grapes, prolonged contact between the juice and skins, stems, or seeds dissolves these materials into the grape must. Tannins isolated from grapes include d-catechin, l-epicatechin, l-epigallocatechin, dl-gallocatechin, and d-epicatechingallate.

5. Vitamins/Minerals. A large variety of vitamins are constituents of grapes (Amerine and Joslyn, 1973a). Ascorbic acid (vitamin C) is present in small but measurable quantities in fresh grapes (1-18 mg/100 g), and partially accounts for their low oxidation-reduction potential. During the ripening process, ascorbic acid levels cycle through two phases of maximal and minimal vitamin C content. Although the skin areas contain more ascorbic acid than the juice, the total amount of ascorbic acid in the juice is greater than in the skin. Other vitamins reported in grape must include vitamin A, thiamin (B1), riboflavin (B2), pyridoxine (B6), pantothenic acid, nicotinic acid, biotin, inositol (discussed

above), p-aminobenzoic acid, choline, and folic acid. The levels of these vitamins increase in grapes during maturation. Grapes are also one of the richest sources of vitamin P (Amerine and Joslyn, 1973a). Vitamin P decreases capillary permeability and fragility (Merck, 1983; Heilbrun et al., 1953; Dressler and Holter, 1982). The vitamin P (citrin group) has chemical and physiological properties of rutin, which is a flavone, and similar compounds (Farris, 1979). Rutin, or quercetin-3-rutinoside, occurs naturally in tobacco leaves (Merck, 1983; Heilbrun et al., 1953).

The ash content of grapes is 0.2-0.6 percent of the fruit's fresh weight (Amerine and Joslyn, 1973a). Potassium, sodium, calcium, phosphates, sulfates, chlorides, and iron as carbonates or oxides compose most of the ash. Fresh must contains 1-30 ppm iron as free ferrous ions and, to a lessor extent, as ferrous complexes and ferric ions. Tartaric, citric, and malic acids form complexes with iron. The ratio of ferric ions (mainly in complex form) to ferrous ions (mainly in free form) depends on the must's state of oxidation. Copper, aluminum, manganese, zinc, cadmium, boron, rubidium, lead, and arsenic levels (traces to 50 mg/L) in grape must reflect the levels of the metals in the plants' soil (Amerine and Joslyn, 1973a). Similarly, the soil conditions and the levels of inorganic anions influence the levels of phosphate, sulfate, chloride, bromide, iodide, and fluoride in grape must.

6. Enzymes. The enzyme complex of grapes is localized primarily in the skin, but can be found to a lesser extent in the pulp (Amerine and Joslyn, 1973a; Amerine et al., 1972a, b). Settling, centrifuging, or fining musts greatly reduces their enzyme content. In addition, heat, tannins, and sulfur dioxide can inactivate must enzymes. The enzyme complex consists of oxidative enzymes (polyphenoloxidase and peroxidase), catalase, invertase (sucrase), tannase, pectic enzymes, ascorbase, acid phosphatases, dehydrases, esterases, and proteases.

Polyphenoloxidase is the main oxidizing enzyme in grapes, and has the greatest activity in the skin. It is a copper-containing enzyme (4 atoms copper/molecule), with a molecular weight of approximately 100,000. Enzymatic oxidation involves the conversion of o-phenol groups to yellow or red quinones, which react with more oxygen to produce brown-colored condensation products. Small amounts of sulfur dioxide (10-50 ug/L) inhibit polyphenoloxidase activity.

Pectins are high molecular weight, hydrocolloidal substances (molecular weight 20,000-100,000), consisting of partially methoxylated galacturonic acids joined in long chains (Hawley, 1982; Merck, 1983). They occur in grapes and a variety of other fruits. The pectin-hydrolyzing enzymes in grape must prevent the pectins from jelling at room temperature and facilitate clarification of the juice. (Amerine and Joslyn, 1973a). Three types of pectic enzymes

are present in grapes: 1) esterases and hydrolases, which split off methyl ester groups from polygalacturonic acid chains, 2) polygalacturonases, which hydrolyze α-1,4-glycosidic linkages in pectin polyuronides, and 3) pectate lyases, which split α-1,4-glycosidic bonds by a transelimination mechanism.

Tannins are complex mixtures of polyphenolics containing primarily gallic acid (3,4,5-Trihydroxybenzoic acid) or other phenol derivatives such as resorcinol, phloroglucinol, or pyrogallol combined with sugars (glucosides) (Halverson and Panzer, 1980). Fruits, including grapes, are a source of tannins (Considine and Considine, 1983b; Amerine and Joslyn, 1973a). Two classes of tannins are: 1) hydrolyzable tannins, which are normally derived from wood extracts and hydrolyzed to yield primarily a carbohydrate moiety (glucose) and gallic or ellagic acid, and 2) condensed tannins, which are derived from grapes, do not contain carbohydrates, and are thought to be polymers of catechin or flavan-3,4-diols (Amerine et al., 1972a; Amerine and Joslyn, 1972a). Condensed tannins can also undergo hydrolysis. The ability of tannins to precipitate proteins is the reason for their astringent (bitter taste and hemostatic action) and antiseptic properties (Sollman, 1964; Singleton and Noble, 1976). They also possess floculant activity, i.e., function as settling aids which enhance the formation of aggregates

(Halverson and Panzer, 1980). The level of tannase activity affects all of these processes.

7. Odorous Constituents. The odorous constituents of grapes increase during ripening (Amerine et al., 1972a; Amerine and Joslyn, 1973a). Grape skins are the primary source of aromatic components, which move into the fruit or pulp during the later stages of maturation. Although a large number of organic acids, alcohols, aldehydes, ketones, and esters are proposed to contribute to grape aroma and flavor, only a small number of odorous constituents are thought to be responsible for the characteristic smell and taste of grapes. The most pronounced odor in grapes has a distinct "eastern" or "foxy" aroma, attributed to the ester methyl anthranilate (Amerine et al., 1972a; Amerine and Joslyn, 1973a; Acree,1980). The largest components of Concord grape essence are esters, predominantly ethyl acetate and methyl anthranilate. "Muscat" aroma is ascribed to a variety of terpenes, primarily terpene alcohols including geraniol, terpineol, limonene, and linalool. Other significant volatile constituents of grape essence are 1-phenylethanol, which contributes a rose-like odor; 2-phenylethanol, linalool, ethyl acetate, and isoamyl acetate, which have a fruity note; and vanillin, which has a unique flavor and aroma.

8. Chemical Additives. Additives used in raisin and grape juice are sulfur dioxide, salts which yield sulfur dioxide, and sorbic acid or sorbate. (Amerine and Ough, 1974;

Considine and Considine, 1983a; Halsey et al., 1968). Prohibited additives used as preservatives in raisin and grape juice include cyanide (from the use of potassium ferrocyanide to remove copper and iron) and salicylic, benzoic, monochlor- and monobromacetic acid. The inadvertent introduction of excess pesticide onto the fruit and into the juice is rare.

References

Acree, T.E. (1980), "Flavor Characterization," Kirk-Othmer Encyclopedia of Chemical Technology, 3rd Ed. Vol. 10. Wiley-Interscience, New York, NY, pp 444-55.

Amerine, M.A. (1984), "Wine," Kirk-Othmer Encyclopedia of Chemical Technology, 3rd Ed., Vol. 24, Wiley-Interscience, New York, NY, pp 549-78.

Amerine, M.A., Berg, H.W., and Cruess, W.V. (1972a), "The Composition of Grapes," The Technology of Wine Making, The Avi Publishing Company, Inc., Westport, CT, pp 76-137.

Amerine, M.A., Berg, H.W., and Cruess, W.V. (1972b), "Fining," The Technology of Wine Making, The Avi Publishing Company, Inc., Westport, CT, pp 309-18.

Amerine, M.A. and Joslyn, M.A. (1973a), "Composition of Grapes," Table Wines The Technology of Their Production, U. of California Press, Berkeley, CA, pp 233-90.

Amerine, M.A. and Joslyn, M.A. (1973b), "Use of Sulfur Dioxide." Table Wines The Technology of Their Production, U. of California Press, Berkeley, CA, pp 380-408.

Amerine, M.A. and Ough, C.S. (1974), Wine and Must Analysis, John Wiley & Sons, New York, NY.

Amerine, M.A. and Roessler, E.B. (1976), Wines Their Sensory Evaluation, W.H. Freeman & Company, San Francisco, CA, pp 31-33, 86-92, 195, 200-201.

Andersen, R. A., Lowe, R. and Vaughn, T. A. (1969), Plant Phenols and Polyphenoloxidase In Nicotiana Tabacum During Greenhouse Growth, Field Growth, and Air-Curing. Phytochemistry, 8: 2139-247.

Bilyk, A. and Sapers, G. M. (1985), Distribution of Quercetin and Kaemferol in Lettuce, Kale, Chive, Garlic Chive, Leak, Horseradish, Red Radish, and Red Cabbage Tissues, J. Agric. Food Chem., 33: 226-28.

Bjeldanes, L. F. and Chang, G. W. (1977), Mutagenic Activity of Quercetin and Related Compounds, Science, 197: 577-78.

Bolin, H.R. (1976), Texture and Crystallization Control in Raisins, J. of Food Sci., 41: 1316-319.

Bolin, H.R. and Stafford, A.E. (1980), A Research Note: Fatty Acid Esters and Carbonates in Grape Drying, J. of Food Sci., 45: 754-55.

Bolin, H.R. and Petrucci, V. (1985), A Research Note, Amino Acids in Raisins, J. of Food Sci., 50: 1507.

Brown, J. P. (1980), A Review of the Genetic Effects of Naturally Occurring Flavonoids, Anthraquinoines and Related Compounds, Mutation Res., 75: 243-77.

Buening, M-K., Chang, R. L., Huang, M-T., Fortner, J. G., Wood, A. W., and Conney, A. H. (1981), Activation and Inhibition of Benzo[a]pyrene and Aflatoxin B_1 Metabolism in Human Liver Microsomes by Naturally Occurring Flavonoids, Cancer Res., 41: 67-72.

Buttery, R.G., Seifert, R.M., Ling, L.C., Soderstrom, E.I., and Yerington, A.D. (1981), "Raisin and Dried Fig Volatile Components: Possible Insect Attractants," Quality of Selected Fruits and Vegetables of North America., Teranishi, R. and Barrera-Benitez, H. (eds.), ACS Symposium Series 170, American Chemical Society, Washington, D.C., pp 29-41.

Clingeleffer, P.R. (1984), Effects of time of season, fruit depth, and covering at night when drying on acceptable moisture content of sultana raisins, J.Sci. Fd. Agric., 35: 173-81.

Considine, D.M. and Considine, G.D. (1983a), "Grape," Van Nostrand's Scientific Encyclopedia, 6th edition, Van Nostrand Reinhold Company, New York, NY, pp 1408-410.

Considine, D.M. and Considine, G.D. (1983b), "Tannin," Van Nostrand's Scientific Encyclopedia, 6th edition, Van Nostrand Reinhold Company, New York, NY, p 2754.

Court, W. A. (1986), High-Performance Liquid Chromatography of Tobacco and Tobacco Smoke Components., Recent Advances in Tobacco Science, 12: 143-184. Tobacco Chemists' Research Conference, Knoxville, Tennessee.

Das, M., Khan, W. A., Asokan, P., Bickers, D. R., and Mukhtar, H. (1987), Inhibition of Polycyclic Aromatic Hydrocarbon-DNA Adduct Formation in Epidermis and Lung of SENCAR Mice by Naturally Occurring Plant Phenols. Cancer Res., 47: 767-73.

Dawson, R. F. and Wada, E., (1957), Flavonoids and Depsides of the Green Tobacco Leaf: I. Rutin and Chlorogenic Acid, Tobacco Science, 1: 47-50.

Deschner, E. E., Ruperto, J., Wong, G., and Newmark, H. L. (1991), Quercetin and Rutin as Inhibitors of Azoxymethanol-Induced Colonic Neoplasia, Carcinogenesis, 12(7): 1193-196.

Dressler, H. and Holter, S.N. (1982), "Polyhydroxybenzines," Kirk-Othmer Encyclopedia of Chemical Technology, 3rd edition, Vol. 18, Wiley-Interscience, New York, NY, pp 670-704.

Dunnick, J. K. and Hailey, J. R. (1992), Toxicology and Carcinogenicity Studies of Quercetin, A Natural Component of Foods, Fundam. Appl. Toxicol., 19: 423-31.

Farris, R.E. (1979), "Dyes, Natural," Kirk-Othmer Encyclopedia of Chemical Technology, 3rd edition, Vol. 8, Wiley-Interscience, New York, NY, pp 351-73.

Froggatt, P. (1988), "Additives to Tobacco and Cigarette Papers," Chapter 5, Fourth Report of The Independent Committee On Smoking and Health, London's first report (1975) was referred to as The Hunter Report.

Guadagni, D.G., Stufford, A.E., and Fuller, G. (1975), Taste Thresholds of Fatty Acid Esters in Raisins and Raisin Paste, J. of Food Sci., 40: 780-83.

Halsey, W.D., Shores, L., Blackburn, R.H., and Francis, F. (1968), "Raisin," Collier's Encyclopedia, Vol. 19, Crowell-Collier Educational Corporation (Macmillan Educational Co.), New York, NY, p 655.

Halverson, F. and Panzer, H.P. (1980), "Flocculating Agents," Kirk-Othmer Encyclopedia of Chemical Technology, 3rd edition, Vol. 10, Wiley-Interscience, New York, NY, pp 489-523.

Hashiba, H., Okuhara, A., and Iguchi, N. (1981), Oxygen-dependent browning of soy sauce and some brewed products, Prog. Fd. Nutr. Sci., 5: 93-113.

Hawley, G.G. (1981), The Condensed Chemical Dictionary, 10th edition, Van Nostrand Reinhold Company, New York, NY.

Heath, H.B. (1981), "Grape" and "Raisin," Source Book of Flavors, The Avi Publishing Company, Inc., Westport, CT, pp 189, 193.

Heilbron, I., Banbury, H.M., Cook, A.H., Jones, E.R.H., Halsall, T.G., and Pollock, J.R.A. (1953), Dictionary of Organic Compounds, Oxford University Press, New York, NY.

Herrmann, K. (1964), Über die phenolischen Inhaltsstoffe des Tabaks und des Tabakrauches, Beitrage zur Tabakforschung, 2(5): 159-79.

Herrmann, K. (1976), Flavonols and Flavones in Food Plants: A Review, J. Food Technol., 11: 433-48.

Hertog, M. G. L., Hollman, P. C. H., and Venema, D. P. (1992), Optimization of a Quantitative HPLC Determination of Potentially Anticarcinogenic Flavonoids in Vegetables and Fruits. J. Agric. Food Chem., 40: 1591-598.

Horowitz, R. M. (1981), "Flavonoids, Mutagens, and Citrus, " In: Quality of Selected Fruits and Vegetables of North America, Teranishi, R. and Barrera-Benitez, H. (eds.). ACS Symposium Series 170, American Chemical Society, Washington, DC, pp 43-59.

Huang, M-T., Wood, A. W., Newmark, H. L., Sayer, J. M., Yagi, H., Jerina, D. M., and Conney, A. H. (1983), Inhibition of the Mutagenicity of Bay-Region Diol-Epoxides of Polycyclic Aromatic Hydrocarbons by Phenolic Plant Flavonoids, Carcinogenesis, 4(12): 1631-637.

Kallianos, A. G., and Means, R. E., and Mold, J. D. (1967), Effect of Nitrates in Tobacco on the Catechol Yield in Cigarette Smoke, Tobacco Science, 12: 125-29.

Kato, R., Nakadate, T., Yamamoto, S., and Sugimura, T. (1983), Inhibition of 12-O-tetradecanoylphorbol-12-acetate-Induced Tumor Promotion and Ornithine Decarboxylase Activity by Quercetin: Possible Involvement of Lipoxygenase Inhibition., Carcinogenesis, 4(10): 1301-305.

Liu, L. and Castonguay, A. (1991), Inhibition of the Metabolism and Genotoxicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) In Rat Hepatocytes by (+)-Catechin, Carcinogenesis, 12(7): 1203-208.

Mazaki, M., Ishii, T., and Uyeta, M. (1982), Mutagenicity of Hydrolysates of Citrus Fruit Juices, Mutation Res., 101: 283-91.

Merck Index (1983), 10th Edition, Merck and Co., Inc., Rahway, NJ.

Oszmianski, J. and Lee, C.Y. (1990), Inhibition of polyphenol oxydase activity and browning by honey, J. Agric. Food Chem., 38: 1892-895.

Pamukcu, A. M., Yalciner, S., Hatcher, J. F., and Bryan, G. T. (1980), Quercetin, A Rat Intestinal and Bladder Carcinogen Present in Braken Form (Pteridium aquilinum), Cancer Res., 40: 3468-472.

Ramshaw, E.H. and Hardy, P.J. (1969), Volatile compounds in dried grapes, J. Sci. Fd. Agric., 20: 619-21.

Singleton, V.L. and Noble, A.C. (1976), "Wine Flavor and Phenolic Substances," Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors, Charalambous, G. and Katz, I. (eds.), ACS Symposium Series 26, American Chemical Society, Washington, D.C., pp 47-70.

Snook, M. E. and Chortyk, O. T. (1982), An Improved Extraction-HPLC Method for Tobacco Polyphenols, Tobacco Science, 26: 25-29.

Sollmann, T. (1964), Manual of Pharmacology and Its Applications to Therapeutics and Toxicology, W.B. Saunders, Philadelphia, PA, pp 1-2, 158-60.

Spanos, G. A. and Wrolstad, R. E. (1992), Phenolics of Apple, Pear, and White Grape Juices and Their Changes with Processing and Storage-A Review. J. Agric. Food Chem., 40: 1478-487.

Stich, H.F., Rosin, M.P., Wu, C.H., and Powrie, W.D. (1981), Clastogenic activity of dried fruits, Cancer Letters, 12: 1-8.

Stolz, D.R., Stavric, B., Stapley, R., Klassen, R., Bendall, R. and Krewski, D. (1984), Mutagenicity screening of foods, II, results with fruits and vegetables, Environ. Mutagenesis, 6: 343-54.

Vrijsen, R., Michotte, Y., and Boeye, A. (1990), Metabolic Activation of Quercetin Mutagenicity, Mutation Res., 232: 243-48.

Wargovich, M. J., Eng, V. W. S., Newmark, H. L. (1985), Inhibition by Plant Phenols of Benzo[a]pyrene-Induced Nuclear Aberrations in Mammalian Intestinal Cells: A Rapid In Vivo Assessment Method. Fd. Chem. Toxic., 23(1): 47-49.

Zane, A. and Wender S. H. (1963), Pyrolysis Products of Rutin, Quercetin, and Chlorogenic Acid, Tobacco Science, 7: 21-23.