nilate, we would speculate that they are isomers of the methyl anthranilate.

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Aroma Quality Evaluation of Tomatoes, Apples, and Strawberries

P. Dirinck,* L. Schreyen, and N. Schamp

For aroma quality evaluation of fresh fruits and vegetables the procedure used for isolation of the volatiles from the food matrix is of the highest importance. Representative isolation of tomato, apple, and strawberry volatiles is performed by adsorption of the volatiles, liberated while macerating the material, on Tenax. Injection via reconcentration in a cooling trap and gas chromatographic–mass spectrometric analysis showed chromatograms, which when compared with other isolation procedures had a simpler composition with higher amount of organoleptic important compounds. However, a major problem of the described procedure was the contamination of the chromatogram of aroma compounds by organic air pollutants.

Until recently the major concern in quality of fresh fruits and vegetables has been focused on external appearance attributes such as color, size, shape, and external defects. However, due to several reasons (such as excessive production; selection of varieties for optimal external quality, optimal production, and resistance to infections, without concern for flavor quality; longer storage times, etc.) problems have arisen concerning the flavor of several economically important fruits and vegetables and a decreasing flavor quality has been observed. The first step in solving this problem is the establishment of a method to judge the organoleptic quality of fresh fruits and vegetables on an objective basis. Indeed, in dialogue with cultivators one could, e.g., select varieties for optimal flavor characteristics in order to come to an improved flavor of economically important fruits and vegetables.

Flavor quality of fruits and vegetables is a combination of an impression on the tongue (taste), mainly determined by the acid–sugar ratio and an impression on the nose (aroma), due to the volatile organic compounds. From these two analyses the aroma analysis is the most difficult to perform and flavor quality evaluation should concentrate on the study of the volatile organic compounds, which determine aroma. Complex mixtures of volatile organic compounds can be analyzed by high-resolution capillary gas chromatography. However, an important aspect is the isolation of the volatile organic compounds from the food matrix. For flavor quality evaluation it is important to isolate a mixture of volatiles, which is representative for the mixture, that we unconsciously send to the nose while eating. Isolation procedures such as steam distillation and solvent extraction are useless for that purpose. Classical head space analysis (10–20 mL sample injection) is not compatible with high-resolution capillary gas chromatography and preconcentration of the volatiles is necessary before analysis. We wish to report on a method for head-space analysis by collection of the volatiles (liberated while macerating the material) on Tenax, injection via reconcentration in a cooling trap and a capillary gas chromatographic–mass spectrometric analysis. The selection of Tenax as adsorbing agent has been inspired by pollution chemistry (Bertsch et al., 1974) and the injection system is an adaption of the system described by Flath et al. (1969a).

Up to now investigations have been concentrated on tomatoes, apples, and strawberries. Fundamental studies with emphasis on component identification have been performed by several laboratories: apples (Schultz et al., 1967; Flath et al., 1967; Drawert et al., 1969; Flath et al., 1969b), tomatoes (Schormüller et al., 1969; Viane et al., 1969; Kazeniac and Hall, 1970; Buttery et al., 1971), strawberries (Winter and Willhalm, 1964; McFadden et al., 1965; Tressl et al., 1969; Black et al., 1971). According to Flath the components directly associated with the characteristic apple-like aroma are: ethyl 2-methylbutyrate, *n*-hexanal, and *trans*-2-hexenal (Flath et al., 1967). Also strawberry flavor has been associated with several volatile organic esters (Black et al., 1971). Several compounds have been suggested to be important to fresh tomato flavor: *trans*-2-hexenal (Kazeniac and Hall, 1970), *n*-hexanal (Kazeniac and Hall, 1970), *cis*-3-hexen-1-ol (Kazeniac and Hall, 1970), β-ionone (Buttery et al., 1971), *trans,trans*-2,4-decadienal (Buttery et al., 1971), and especially 2-isobutylthiazole (Viane et al., 1969; Kazeniac and Hall, 1970; Buttery et al., 1971), and *cis*-3-hexenal (Kazeniac and Hall, 1970; Buttery et al., 1971; Guadagni et al., 1972).

The occurrence and concentration of these organoleptic important compounds in a chromatogram of a representatively isolated flavor mixture is a suitable method for flavor quality evaluation (Dirinck et al., 1975, 1976). In the present study the new isolation procedure is illustrated for apples, tomatoes, and strawberries and its advantages

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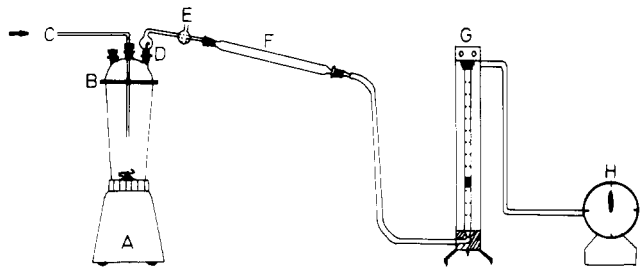

Figure 1. Sampling apparatus.

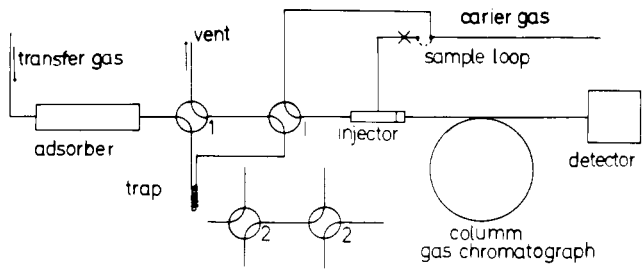

Figure 2. Pre-injection trapping system.

and limitations are discussed. Objective flavor quality evaluations of different samples of fresh fruits and vegetables (different varieties, flavor quality in function of storage time, etc.), based on the occurrence and concentration of organoleptic important compounds in the chromatograms obtained by the described isolation procedure, and relation with flavor quality evaluations by a taste panel will be published separately.

EXPERIMENTAL SECTION

Description of Sampling Apparatus. The sampling apparatus is presented in Figure 1. A commercial blender A (Braun MX32, Germany), in which all plastic parts had been changed by Teflon, was adapted with a glass flange B and fitted with a three-necked flange cover. During sampling helium gas was supplied through C. The cover was fitted with a splash head D with dump valve E, which was necessary to exchange the contaminated air inside the blender (volatile organic air pollutants) by helium before sampling. The splash head E was connected to the adsorber F. The helium gas flow, regulated by a fine metering valve, was measured by the flow meter G and the total volume of the gas sample by the wet-testmeter H.

Adsorbent. For its thermal stability and feature not to adsorb water, a polyaromatic polar polymer, Tenax GC 60/80 mesh (Applied Science Lab., Inc., State College, Pa.) was selected as an ambient temperature adsorbent and was packed in a relatively large glass adsorption column (i.d., 1.6 cm; length, 10 cm) with an adsorbent content of 5 g. For quantitative analysis helium passed through the adsorption column with a flow rate of 30 L/h during 15 min. Loaded columns were closed and could be stored for a long period without loss of sample or changes in the composition.

Sampling Procedure. About 250 g of fruits or vegetables were brought into the blender (stalks were removed and apples were quartered), which was closed as hermetically as possible and provided with the features described above. Before disintegration was started, the inside space of the blender was washed with helium through dump valve E during 10 min. After switching valve E, disintegration of the fruits was started slowly (the rotation speed of the propellers was regulated by a Vareac), and adsorption was continued for 15 min, while further disintegrating the material.

Sample Injection. Sample injection was performed via reconcentration of the sample in a cooling trap. The adsorber was connected to the device illustrated in Figure 2. Desorption was effected by heating the adsorber for 60 min at 220 °C, while helium was flowing through at a flow rate of 50 mL per minute. The sample was collected in a trap cooled with liquid nitrogen. Because of the high water concentration in the sample and the risk of clogging the trapping system by ice formation, the elution temperature of 220 °C had to be reached by gradually warming up the adsorber. For injection the liquid nitrogen was replaced by an oil bath at 220 °C while the valve system was switched from collecting position 1 to injection position 2.

Gas Chromatography–Mass Spectrometry. Due to the minute amount of substances, even after concentration, identification could only rely on mass spectrometry, assisted by retention indices at linear temperature programmation. For the latter purpose a mixture of *n*-alkanes in carbon disulfide was injected on the adsorber before transferring the sample to the pre-column trap. The GC–MS apparatus consisted of a Varian 1200 gas chromatograph linked to a MS 30 double-beam mass spectrometer (A.E.I., Manchester) via a membrane separator. As due to the water content of the sample, the monitogram was disturbed during an important part of the analysis, an effluent splitter was installed to send about 75% of the total gas flow to the separator and 25% to the F.I.D.

Make-up gas was added at the column exit in order to obtain an optimum gas flow of about 20 mL of He/min through the separator. Open tubular glass columns (i.d., 0.6 mm) were coated with methyl silicone oil SE-30 by the static coating procedure (Bouche and Verzele, 1968). To obtain lengths of up to 400 m, several columns were connected by means of a shrinkable polymer (Krimpkous, Raychem). A glass insert was connected directly to the column and trapped organics were transferred unsplit into the column.

Operating conditions for GC-MS were: 388 × 0.6 mm i.d. glass column, coated with SE-30; linear temperature programming from 20 to 220 °C at 2 °C/min; carrier gas He, 6 mL/min and make-up gas to 20 mL/min; temperatures: injector, 220 °C; separator oven and interconnecting lines, 200 °C; ion source, 200 °C; ion source pressure, 10^{-5} mmHg; trap current, 300 μA; filament voltage, 70 V; scan speed, 3 s/decade.

RESULTS AND DISCUSSION

Several gas chromatographic analyses of the same batch of fruits or vegetables indicated the procedure to be reproducible. As was proven by the use of two consecutive adsorbers at low flow rate (30 L/h and lower), adsorption was above 90% for all substances with boiling point over 0 °C, except when concentrations were too high (*k* values at room temperature on Tenax are very dependent on concentrations). However, though adsorption was nearly quantitative, there was loss of volatiles with programmed temperature retention indices above 1100, which was due to condensation of the higher boiling compounds in the injection system, while transferring the sample before injection.

The described procedure allowed relatively fast isolation and analyses of a flavor mixture, which was representative for the mixture of volatiles, we unconsciously send to the nose while macerating our food. However, besides these important advantages, a major problem of the method consisted of the contamination of the chromatogram of flavor compounds by volatile organic air pollutants. Several measures were taken to avoid this contamination:

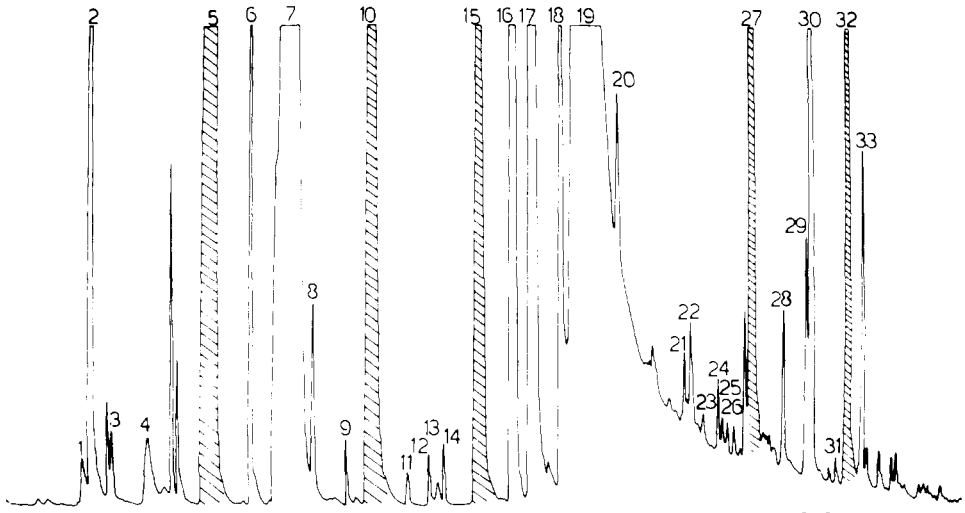

Figure 3. Gas chromatogram of a tomato sample. Organoleptic important compounds are shaded.

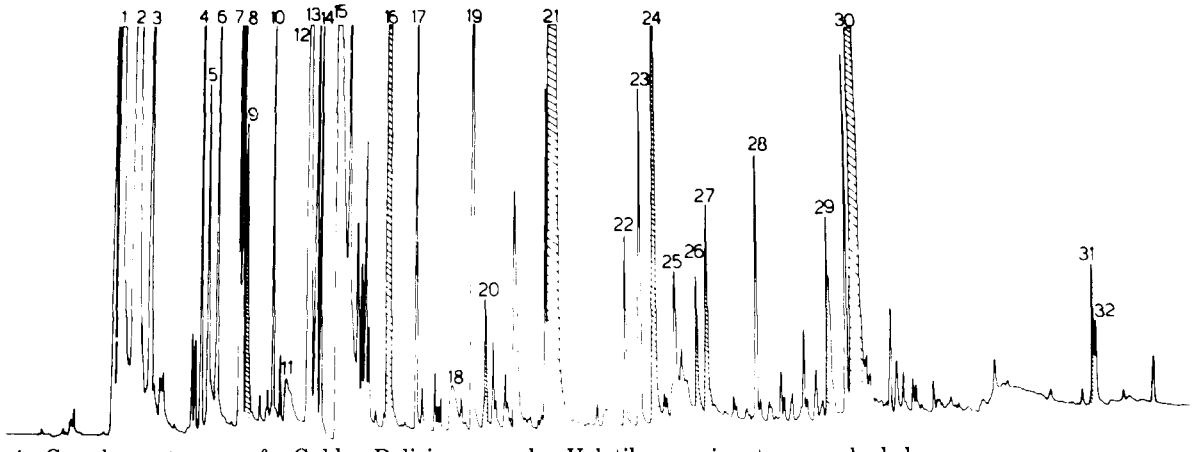

Figure 4. Gas chromatogram of a Golden Delicious sample. Volatile organic esters are shaded.

all plastic parts in the blender were changed by Teflon; before sampling all glass blender parts were cleaned in Decontamin (Apiel, Brussels), dried in an oven, and cooled under nitrogen; before adsorption was started the inner space of the blender was washed by pure helium; samples were taken outside the laboratory in a "clean" room. Although the precautions mentioned reduced the described contamination considerably, it could not completely be eliminated and was possibly due to retrodiffusion of air pollutants into the inside space of the blender during sampling. So even for routine analyses as in quality control GC–MS coupling is an obligation.

As an illustration of the described isolation procedure analyses of tomato, apple, and strawberry samples are given below. Gas chromatographic–mass spectrometric analyses as a tool for objective flavor evaluations of different sorts of apples, tomatoes, and strawberries and relations with panel evaluations will be published later.

Tomatoes. A typical gas chromatogram of a tomato sample is given in Figure 3. The identified compounds are presented in Table I, with their peak number of Figure 1. Organoleptic important compounds are shaded and contaminating organic air pollutants are indicated. With subtraction of the contaminating air pollutants, the GLC pattern of the representatively isolated tomato sample is rather simple. Aromagrams, taken by different sniffers, indicated valeronitrile, *n*-hexanal, *trans*-2-hexenal, 2-methyl-2-hepten-6-one, and 2-isobutylthiazol as important contributors to the fresh tomato flavor. Compared with other isolation procedures (Dirinck et al., 1976; Buttery et al., 1971), the described isolation procedure shows a simpler chromatogram, in which the contributory compounds also occur in a relatively high amount.

Table I. Compounds Identified in Figure 3

1. 2-Methylpropan-1-ol	18. 1-Hexanol
2. 3-Methylbutanal	19. Unidentified
3. Benzene[a]	20. *n*-Nonane[a]
4. 1-Butanol	21. α-Pinene
5. Valeronitrile	22. Benzaldehyde
6. 2-Methyl-2-butenal	23. Propylbenzene[a]
7. 3-Methylbutan-1-ol	24. Isomeric ethylmethylbenzene[a]
8. Toluene[a]	25. Isomeric ethylmethylbenzene[a]
9. Pyridine[a]	26. Isomeric trimethylbenzene[a]
10. *n*-Hexanal	27. 2-Methyl-2-hepten-6-one
11. Tetrachloroethylene[a]	28. Isomeric trimethylbenzene[a]
12. Isomeric hexenol	29. Dichlorobenzene[a]
13. *cis*-3-Hexenal	30. Unidentified
14. 2-Methylpentan-1-ol	31. Isomeric trimethylbenzene[a]
15. *trans*-2-Hexenal	32. 2-Isobutylthiazole
16. Ethylbenzene[a]	33. β-Phellandrene
17. *m*- and *p*-Xylene[a]	

[a] Organic air pollutants: compounds which were also present in laboratory air samples are indicated as air pollutants.

Apples. A typical gas chromatogram of a Golden Delicious sample is given in Figure 4 and the corresponding identifications are gathered in Table II.

Besides air contaminants, a few alcohols, and terpenes, the chromatogram is composed of several volatile organic

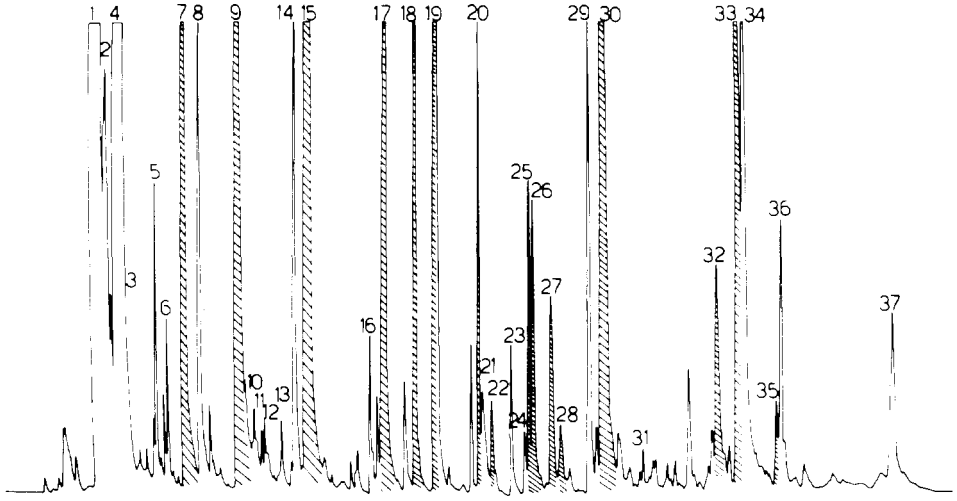

Figure 5. Gas chromatogram of a strawberry (variety Gorella) sample. Organoleptic important esters are shaded.

Table II. Identification of Figure 4

1. Acetone[a]	17. Methylcyclohexane[a]
2. Diethyl ether[a]	18. 3-Methylbutan-1-ol
n-Pentane[a]	19. Toluene[a]
3. Dichloromethane[a]	20. 2-Methyl-1-propyl acetate
4. 2-Methylpentane[a]	21. 1-Butyl acetate
5. 2-Methylpropanal	22. Ethylbenzene[a]
6. 3-Methylpentane[a]	23. *m*- and *p*-Xylene[a]
7. *n*-Hexane[a]	24. 3-Methyl-1-butyl acetate
8. Chloroform[a]	25. *o*-Xylene[a]
9. Ethyl acetate	26. 1-Butyl propionate
10. Methylcyclopentane[a]	27. 1-Pentyl acetate
11. 2-Methylpropan-1-ol	28. α-Pinene
12. Benzene[a]	29. 1-Butyl butyrate
13. Tetrachloromethane[a]	30. 1-Hexyl acetate
14. Cyclohexane[a]	31. 1-Hexyl butyrate
15. 1-Butanol	32. Estragol
16. 1-Propyl acetate	

[a] Organic air pollutants.

Table III. Compounds Identified in Figure 5

1. Acetone[a]	20. 1-Methyl-1-ethyl butyrate
2. Diethyl ether[a]	21. *trans*-2-Hexenal
3. Dichloromethane[a]	22. Ethyl 2-methyl-butyrate
4. Carbondisulfide[a]	23. Ethylbenzene[a]
5. 2-Methylpentane[a]	24. *p*- and *m*-Xylene[a]
6. 3-Methylpentane[a]	25. 3-Methyl-1-butyl acetate
7. Mixture	26. 2-Methyl-1-butyl acetate
Ethyl acetate	27. Mixture
Chloroform[a]	Styrene[a]
n-Hexane[b]	Methyl 4-methyl-pentanoate
8. Methyl propionate	28. Mixture
9. Mixture	*o*-Xylene[a]
Benzene[a]	1-Propyl butyrate
1-Methyl-1-ethyl acetate	29. *n*-Nonane[b]
10. Tetrachloromethane[a]	30. Methyl hexanoate
11. Methyl 2-methylpropionate	31. α-Pinene
12. 2-Methylhexane[a]	32. Ethyl hexanoate
13. 3-Methylhexane[a]	33. 1-Hexyl acetate
14. *n*-Heptane[b]	34. *n*-Decane[b]
15. Methyl butyrate	35. 2-Pentyl butyrate
16. Toluene[a]	36. Limonene
17. Methyl 2-methylbutyrate	37. *n*-Undecane[b]
18. Ethyl butyrate	
19. Mixture	
1-Butyl acetate	
n-Octane[b]	

[a] Organic air pollutants. [b] Before injection *n*-alkanes were injected on the column for retention index determination.

esters, which with no doubt are the most important contributory compounds to the Golden Delicious flavor. Flavor quality of different apple varieties, evaluated by a taste panel could be related to the volatile ester composition of their essential apple oil, isolated by head-space condensation (Dirinck et al., 1975). Also in the described isolation procedure, which allows a faster representative isolation of volatiles than the head-space condensation procedure (Dirinck et al., 1976), several organoleptic important esters are absent in the Golden Delicious chromatogram when compared with other varieties, indicating the poor aroma characteristics of the Golden Delicious variety.

Strawberries. Table III lists the compounds identified in a strawberry sample (variety Gorella) with the corresponding peak numbers of Figure 5. Disregarding the air pollutants, the composition of the chromatogram in Figure 5 indicates that also the fresh strawberry flavor is nearly completely composed of volatile organic esters.

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Dihydrochalcone Sweeteners. Synthesis and Sensory Evaluation of Sulfonate Derivatives

Grant E. DuBois,* Guy A. Crosby, Rebecca A. Stephenson, and Robert E. Wingard, Jr.*

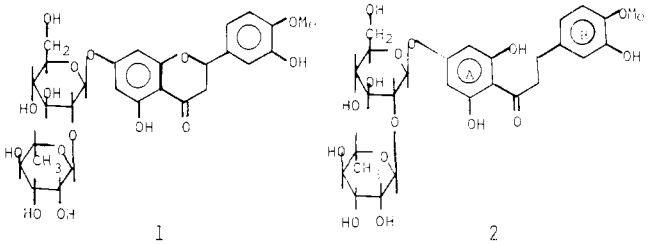
Fifteen sulfonate analogues of hesperetin dihydrochalcone (DHC), the aglycone portion of the intensely sweet glycosidic flavonoid neohesperidin DHC, were prepared and subjected to sensory analysis. Three distinct synthetic routes, the most general of which involves the regioselective alkylation of hesperetin at the 7 position followed by alkaline hydrogenation, were developed for the preparation of these materials. The simple linear 4-*O*-sulfoalkyl-DHC derivatives exhibited taste properties comparable to neohesperidin DHC. These sulfonates were found, however, to exhibit a slow taste onset followed by a lingering aftertaste as appears typical for DHC sweeteners. Taste–timing properties are discussed from the viewpoint of modern sensory theory, and a model, speculating on the various aspects of the DHC molecule responsible for the observed taste, has been developed.

Several years ago Horowitz and Gentili reported that the peels of oranges, lemons, and grapefruit contained a number of flavonoid derivatives which could, by way of a simple chemical modification, be converted into a new class of sweet compounds (1963, 1969). They found, for example, that the flavanone rhamnoglucoside, neohesperidin (1), the predominant bitter principle of the Seville orange rind, readily afforded the intensely sweet dihydrochalcone (DHC) derivative 2 upon alkaline hydrogenation. Similar results were reported for other flavanones which are conjugated with a β-neohesperidose sugar residue through the 7 phenolic hydroxyl position.

Neohesperidin DHC (2) has been indicated to be an attractive sweetener from a safety viewpoint (Booth and Robbins, 1968; Booth et al., 1973; Gumbmann et al., 1975) and it has been shown that the material can be prepared in a reasonably economic fashion on an industrial scale (Robertson et al., 1974). On the other hand, a serious problem is derived from the fact that the intense sweetness of DHC 2 is rather slow in onset and lingers considerably. These poor taste–timing characteristics render the sweetener unsuitable for use in most food products (Inglett et al., 1969).

It has been known for some time that hesperetin dihydrochalcone (3), which is the poorly soluble aglycone portion of 2, is sweet (Horowitz, 1964; Rizzi and Neely, 1973). We recently reported that water-soluble derivatives of 3 could be prepared by attaching carboxyalkyl chains to the hydroxyl group at position 4 (DuBois et al., 1977a). These compounds, although intensely sweet, were found to suffer from the same poor taste–timing characteristics which affected DHC 2. Additionally, these carboxylate-derived sweeteners were found to have limited solubility in the pH range of beverage systems.

We report here the preparation and taste properties of 15 sulfonated hesperetin dihydrochalcone derivatives.



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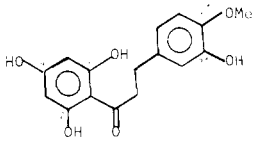

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These compounds were prepared with the expectation that the ionic sulfonate group would lead to high water solubility throughout the useful pH range and might, as the result of the increase in hydrophilic character, provide DHC sweeteners with improved taste–timing characteristics.

MATERIALS AND METHODS

Sensory Evaluation Procedure. All new compounds were given a preliminary taste evaluation by sampling a dilute aqueous solution. Compounds of further interest were submitted to a panel of six trained judges for sensory analysis. The panel evaluated the overall taste intensity of aqueous solutions of the materials and characterized the basic tastes present using standard psychophysical procedures (Acton et al., 1970).

The panel members were trained in the recognition of the basic tastes of sweet (as sucrose), sour (as citric acid), salty (as NaCl), and bitter (as quinine sulfate), as well as in the technique of magnitude estimation which consists of ranking the total intensity of a test solution relative to a sucrose standard. The tasting procedure consisted of giving panel members samples in coded beakers along with

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