Carrot Flavor. Sugars and Free Nitrogenous Compounds in Fresh Carrots

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Preparative ion exchange chromatography of the 80% ethanol extract of fresh Imperator carrots separated it into two fractions—sugars and free nitrogenous compounds. Twenty ninhydrin-reacting compounds were quantified and identified as aspartic acid, α-alanine, serine, glutamic acid, glucosamine, arginine, valine, threonine, isoleucine, phenylalanine, tyrosine, histidine, phosphoethanolamine, leucine, β-alanine, cystine, glycine, taurine, methionine sulfoxide, and proline. Four free sugars and six sugar phosphates were positively identified as sucrose, β-glucose, α-glucose, fructose, glucose-6-phosphate, fructose-6-phosphate, glucose-1-phosphate, nucleoside monophosphate, nucleoside diphosphate, and nucleoside triphosphate. The contribution of these compounds to the delicate flavor of carrots is discussed.

Factors involved in the deterioration of the quality of carotene-rich vegetables have been the focal study of several investigations (Falconer *et al.*, 1964; Farine *et al.*, 1965; Mackinney *et al.*, 1958). Carrots were chosen as representative of this group.

The characteristic aroma of raw carrots (Heatherbell and Wrolstad, 1971b; Heatherbell *et al.*, 1971) and processed carrots (Buttery *et al.*, 1968; Heatherbell *et al.*, 1971) is due mostly to their volatile components. Reducing sugars and amino acids undergo structural rearrangement upon processing, producing volatile and nonvolatile compounds of characteristic aroma and flavor notes (Hodge, 1967; Self, 1967). The possibility that characteristic raw carrot aroma could be generated from precursor compounds when reacted with flavor-forming enzymes was explored by Heatherbell and Wrolstad (1971a), who reported that their experimental results were inconclusive possibly due to complex interactions in carrot aroma production.

A number of investigations have indicated that free sugars and free amino acids vary considerably among carrot varieties and were influenced by environmental, agricultural, and storage conditions (Sistrunk *et al.*, 1967). Sucrose, glucose, fructose, and maltose are considered to be the common sugars of carrots. In fresh carrots, total sugar content ranges are reported from 3.46 to 10.74% (Lee, 1951; Meyers and Croll, 1921; Rygg, 1945; Watt *et al.*, 1963; Winton and Winton, 1935). There are reports (Falk, 1919; Platenius, 1934; Shutt, 1910) on the isolation of one or more sugars from fresh carrots. Recently, Otsuka and Take (1969) reported the presence of 0.379 g of glucose, 2.08 g of maltose, and 2.41 g of sucrose per 100 g of fresh carrots grown in Japan. The only references on the amino acids of carrots are those of Bourke *et al.* (1967) and Otsuka and Take (1969). Bourke *et al.* (1967) reported the presence of aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine in fresh carrots grown in the USA. In addition to these amino acids, Otsuka and Take (1969) reported the presence of cystine, lysine, tryptophan, histidine, arginine, and taurine in carrots grown in Japan.

The object of the present study is to obtain a more complete picture of the free amino acids, amines, free sugars, and sugar phosphates present in carrots which constitute either the components responsible for taste *per se* (Kirimura *et al.*, 1969; Moncrieff, 1967; Solms, 1969) or carrot flavor precursors through participation in nonenzymatic browning (Hodge, 1967; Self, 1967).

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EXPERIMENTAL SECTION

Imperator carrot variety, used in this study, was purchased in California and shipped air freight to Natick Laboratories. Upon arrival, the carrots were stored at 5° for 2 to 4 days.

Extraction of Nitrogenous Compounds and Sugars. Sound carrots were washed by hand in running water and dried by blotting with paper towels (Figure 1). The crowns and tips were discarded. After determining moisture content, about 200 g at a time were weighed, diced, and placed in a heavy duty Waring blender. Sufficient 95% ethanol previously cooled to about −20° was added to the carrot sample to obtain an 80% ethanol solution by volume. The contents were blended for 2 min, and the resulting slurry was transferred quantitatively to an Erlenmeyer flask containing 1 g of calcium carbonate to neutralize organic acids (Association of Official Analytical Chemists, 1970a), thus minimizing sugar hydrolysis. The contents were boiled for 30 min to inactivate the enzymes present. The solvent was decanted and filtered through Whatman no. 41 filter paper. The alcohol content of the filtrate was adjusted to a final concentration of 80% by the addition of 95% ethanol and stored in the dark. The carrot residue was extracted overnight with 80% ethanol in a Soxhlet apparatus (Waldron *et al.*, 1948). The ethanol extracts were combined and concentrated under vacuum at 40°. The concentrate was shell frozen and freeze-dried. The lyophilized extract was stored at −20° in brown jars in a vacuum desiccator over phosphorus pentoxide. The carrot residue, after alcohol extraction, was extracted for 24 hr in Soxhlet apparatus with petroleum ether (bp 30–60°) and chloroform–methanol–water, 12:84:4 (v/v/v), respectively. The solvents used contain 0.01% hydroquinone to prevent oxidation of unsaturated fatty acids. The organic solvent extracts were evaporated to dryness at 40° under vacuum. The residue was blanketed with N_2 gas and stored at −29°.

Moisture Content, Total Nitrogen, and Phosphorus Content. Triplicate samples of fresh carrots were used for moisture content determination by the method of the AOAC (1970c). Total nitrogen content was determined using the Dumas method (AOAC, 1970b) with a Coleman model 29A nitrogen analyzer (Coleman Instruments Division, Perkin-Elmer Corp., Maywood, Ill.). Phosphorus was detected according to the method of Fiske and Subbarow (1925), and the quantitative determination of sugar phosphates was made spectrophotometrically (AOAC, 1970d).

Fractionation of Carrot Ethanolic Extract. An aqueous solution of lyophilized carrot alcohol extract (20 mg/g of resin) was percolated through a regenerated column of AG 50W-X8 (hydrogen-form) resin, 200–400 mesh (Calbiochem, Los Angeles, Calif.). The column was washed with distilled deionized water to remove sugars. The eluate was

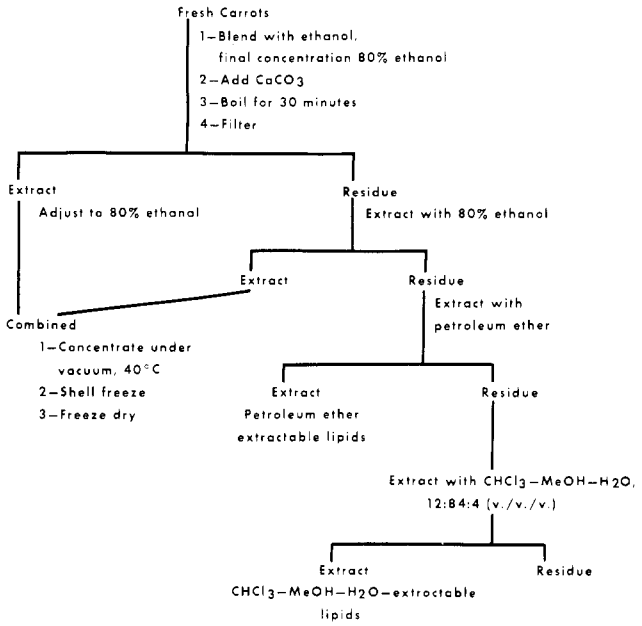

Figure 1. Procedure for preparation of carrot extracts.

shell frozen and freeze-dried, and the resulting sugars were stored in a desiccator under vacuum over P_2O_5 at −29°. The free nitrogenous compounds were desorbed from the column with 2 *N* ammonium hydroxide solution. The eluate was concentrated in a rotary evaporator under vacuum at 40°, shell frozen, freeze-dried, and the lyophilized powder was stored in a desiccator under vacuum over P_2O_5 at −29°.

Amino Acid Analysis. The amino acid fraction was analyzed by ion-exchange chromatography (Moore *et al.*, 1958; Spackman *et al.*, 1958) using a Phoenix Amino Acid Analyzer Model No. K-8000C (Phoenix Precision Instrument Co., Philadelphia, Pa.). Specifications for sample treatment, reagents, resins, columns, temperatures, buffers, and flow rates were those given in the instrument manual. The respective concentrations of the amino acids were calculated by the use of elution times and integration constants of selected individual standards.

Sugar Determination. An aqueous solution of carrot sugar fraction (20 mg/g of resin) was percolated through a column of Dowex 1-X8, chloride form, 200–400 mesh. While sugar phosphates were adsorbed on the resin, free sugars eluted from the resin with 0.001 *M* ammonium hydroxide. The eluate was shell frozen and lyophilized. The adsorbed sugar phosphates on the column were eluted according to the method of Khym and Cohn (1953) and quantitatively determined.

Gas Chromatography of Free Sugars. Free sugars fraction and pure sugar standards were converted to their trimethylsilyl derivatives (Sweeley *et al.*, 1963). A Beckman GC-4 gas chromatograph equipped with dual hydrogen flame detector was used in this work. The gas chromatography conditions are summarized in Table I.

The concentration of individual sugars in carrot extract was estimated from a straight-line correlation between the responses of Infotronics digital integrator model CRS-11 HSPB (Infotronics, Houston, Tex.) and known concentrations of standard sugars.

RESULTS AND DISCUSSION

Upon extraction of 1283.38 g of fresh carrots with 80% ethanol, according to the scheme outlined in Figure 1, 130.66 g of orange-yellowish powder, which amounted to 10.18% of fresh carrots, was obtained. This fraction has a distinctive sweet potato syrup aroma. Group separation of the alcohol extract on ion exchange gave two fractions (*i.e.*, sugars and free nitrogenous compounds) that accounted for 8.91 and 1.31% of fresh carrots, respectively. Moisture content, sugars, free nitrogenous compounds, petroleum ether-extractable lipids, and chloroform–methanol–water-extractable lipids are listed in Table II.

The total nitrogenous compounds fraction obtained by group separation on AG 50W-X8 (hydrogen-form) resin represented 1.37% of fresh carrots. The difference between this figure and the total of the individual nitrogenous compounds listed in Table III (1.31% of fresh carrots) represents peptides and nonprotein nitrogenous compounds which were eluted from the ion exchanger along with the free amino acids, amines, and sugar amines but were not detected on the amino acid analyzer. This assumption is substantiated by the Dumas nitrogen values obtained for the free nitrogenous compounds listed in Table III and for the total nitrogenous fraction isolated by group separation, 12.96 and 14.31%, respectively.

The values reported for petroleum ether-extractable lipid and chloroform–methanol–water-extractable lipid are in agreement with findings of Hanahan and Chaikoff (1947).

The free nitrogenous compounds identified in fresh carrots, along with their concentrations, are recorded in Table III.

The present investigation demonstrates the presence of 20 free nitrogenous compounds in carrots. Thirteen of these compounds, *i.e.*, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine, have been identified and quantified in carrots grown in the USA (Bourke *et al.*, 1967). Except for the absence of glucosamine, phosphoethanolamine, and β-alanine and the presence of tryptophan and lysine in carrots grown in

Table I. Gas Chromatography Conditions

	I	II	III
A. Columns (stainless steel)	6 ft × ⅛ in o.d.	8 ft × ¼ in. o.d.	6 ft × ¼ in. o.d.
Liquid phase	3% SE-30	15% EGS	3% SE-52
Solid phase	60/80 Chrom G, AW, DMCS	80/100 Chrom W, AW, DMCS	80/100 Chrom W, AW, DMCS
B. Temperature, °C			
Injection port	230	160	220
Inlet lines	235	165	230
Detector lines	275	175	275
Detector	275	175	275
Columns	80 to 250, Temp program 4°/min	150 Isothermal	150 to 240, Temp program 3°/min
C. Carrier flow (He)	15 cm^3/min	60 cm^3/min	60 cm^3/min

Japan, Otsuka and Take (1969) reported the same amino acids listed in Table III. Concerning the concentration of each amino acid, some differences were observed between the values reported by Bourke *et al.* (1967), Otsuka and Take (1969), and the present investigation. These variations are anticipated since varieties, climate, soils, fertilization, thinning, cultivation, harvesting, and storage affect the composition of carrots (Bradley *et al.*, 1967).

Aspartic acid, α-alanine, serine, and glutamic acid in the free form are abundant in fresh carrots and account for about 68% of the total free nitrogenous compounds. The basic amino acids, arginine and histidine, amount to 5.23 and 1.23% of the total free nitrogenous compounds, respectively. While threonine and valine account for about 10% of the free nitrogenous compounds present in fresh carrots, the total concentration of sulfur-containing amino acids (*i.e.*, methionine sulfoxide, cystine, and taurine) is less than 1.5%.

The individual concentration of phenylalanine, isoleucine, and tyrosine is less than 2% and that of phosphoethanolamine, leucine, β-alanine, glycine, and proline is less than 1% of the total free nitrogenous compounds in fresh carrots.

Four free sugars and six sugar phosphates were identified in fresh carrots (Table IV).

Sucrose accounts for 44% of the total free sugars content, which amounts to 8.14% of fresh carrots. Fructose occurs in quantity approaching one-fourth of the total reducing sugars in fresh carrots. This finding is in contrast with that of Rygg (1945), who reported that fructose content is one-half the total reducing sugars present in fresh Danvers and Imperator carrots. The reducing sugars (α-glucose, β-glucose, and fructose) amount to 54% of the total free sugars in fresh carrots, which is in agreement with Falk's results (1919). Attempts to identify the unknown sugar (about 0.39% of fresh carrots) were unsuccessful. Its trimethysilyl derivative retention value and co-injection with standards proved it was neither maltose, ribose, inositol, nor galactose. Although an anomeric form of fructose may be a possibility, Sweeley *et al.* (1963) reported that fructose isolated from aqueous solution gave a single peak on SE-52. Our results concerning the absence of maltose in fresh Imperator carrots are in agreement with those of Rygg (1945) and Gawadi (1947). The sugar phosphates amount to 0.8% of fresh carrots.

The overall "flavor sensation" of carrots may be divided into sensations due to "taste" and to "aroma." While the nonvolatile components of carrots (sugars and amino acids) are the taste-bearing compounds, the volatile components are responsible for aroma. Due to the delicate flavor of carrots, the contribution of essential oils may, in our opinion, be small in comparison with that of the nonvolatile, taste-bearing components. The aroma of lyophilized 80% ethanol extract was reminiscent of sweet potato syrup. When the volatile components were extracted with organic solvents (Freon 11, chloroform, and methanol, respectively), the residue was void of aroma, but tasted sweet (Mabrouk and Swift, 1971). The sweetness is due to the sugars and free amino acids present in the carrot alcohol extract. While 87% of the total weight of the free nitrogenous compounds listed in Table II have a flat or sweet flavor and 9% (glutamic acid) have a unique flavor, the rest (4%) have a bitter taste (Solms, 1969). Otsuka and Take (1969) attribute the taste of carrot water extract to the presence of glutamic acid and the buffer action of the other amino acids.

Flavor production is an important result of the nonenzymatic browning reactions occurring during food processing. The particular flavor of cooked carrots varies according to the cooking temperature and the method of cooking; *i.e.*, dry heat or moist heat. The taste and aroma of the products of the browning reaction vary according to the reacting amino acids. The aroma resulting from heating to 100° equimolar solutions of glucose and each one of the following amino acids which are abundant in fresh carrots—aspartic acid, α-alanine, glutamic acid, valine, threonine, serine, and arginine—are reminiscent of rock candy, sweet caramel, caramel, rye bread, chocolate, maple syrup, and buttery notes, respectively (Barnes and Kaufman, 1947; Herz and Shallenberger, 1960; Kiely *et al.*, 1960). Different aromas are produced by the same reaction mixtures when the temperature is raised to 180°.

Table II. Composition of Fresh Carrots

Component	Percentage
Moisture	85.40
80% Ethanol-extractable compounds	10.18
Sugars	8.91
Free nitrogenous compounds	1.31
Petroleum ether-extractable compounds	0.004
Chloroform–methanol–water-extractable compounds	0.13

Table III. Free Nitrogenous Compounds in Fresh Carrots

Nitrogenous compounds	% fresh carrots	% of total identified compounds
Aspartic	0.392	29.95
α-Alanine	0.265	20.23
Serine	0.118	9.03
Glutamic	0.117	8.93
Glucosamine	0.071	5.83
Arginine	0.069	5.23
Valine	0.068	5.18
Threonine	0.063	4.83
Isoleucine	0.024	1.87
Phenylalanine	0.024	1.82
Tyrosine	0.020	1.53
Histidine	0.016	1.23
Phosphoethanolamine	0.013	0.99
Leucine	0.011	0.84
β-Alanine	0.010	0.79
Cystine	0.009	0.69
Glycine	0.007	0.54
Taurine	0.006	0.44
Methionine sulfoxide	0.005	0.34
Proline	0.002	0.15
Total	1.310	100.00

Table IV. Free Sugars and Sugar Phosphates in Fresh Carrots

	% fresh carrots	% total sugars
A. Free sugars	8.17	91.0
Sucrose	3.39	37.9
β-Glucose	1.89	20.8
α-Glucose	1.45	16.2
Fructose	1.05	11.7
Unknown	0.39	4.4
B. Sugar phosphates	0.80	9.0
Glucose 6-phosphate	0.23	2.5
Fructose 6-phosphate	0.16	1.8
Nucleoside monophosphate[a]	0.16	1.8
Glucose 1-phosphate	0.14	1.6
Nucleoside diphosphate[a]	0.07	0.8
Nucleoside triphosphate[a]	0.04	0.5
Total	8.97	100

[a] The colorimetric method used does not distinguish one nucleotide from another.

In conclusion, this investigation gives a clear picture of the nonvolatile chemical constituents primarily responsible for the taste of fresh carrots. Work is in progress to assess the flavor notes resulting from heating the essential oils—free 80% ethanol extract and evaluate the contribution of the resulting volatile and nonvolatile compounds to carrot flavor.

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Nonvolatile Acids in Pineapple Juice

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The nonvolatile organic acids were extracted from summer and winter pineapple juice, separated by tlc, and identified as citric, malic, malonic, glycolic, tartaric, and galacturonic. Gas–liquid chromatography of methyl esters of the acids confirmed the presence of citric, malic, and malonic acids and detected, in addition, succinic acid; glc of TMS derivatives revealed the presence also of phosphoric acid. Seasonal variations in total acidity and relative amounts of citric, malic, and succinic acids were determined.

Pineapple juice has become one of the important processed products of pineapple. In 1970, over 8 million cases of pineapple juice were produced (Hawaii Department of Agriculture, 1972).

The acidity of the juice has been noted to vary with the season of harvest; fruit harvested in the winter has higher acidity and that harvested in summer has lower acidity (Mehrlich, 1961). The nature and amounts of organic acids in pineapple have been studied by a number of workers. Using the method of ester distillation, Nelson (1925) found that the acids in pineapple were 87% citric and 13% l-malic. In "The Pineapple," by Collins (1960), it was stated that the ratios of citric, malic, and ascorbic were 80:20:2; he reported the amounts were 10.87–13.98 mequiv of citric acid/100 g, 2.93 mequiv of malic acid/100 g, and 0.045–0.114 mequiv of ascorbic acid/100 g. Mehrlich (1961) described the 1949–1950 pineapple juice pack as averaging 14.60 mequiv of acid/100 g of juice and that the total acids varied from 12.8 to 28.4 mequiv. Citing unpublished data by Clark from 1939, Mehrlich stated that citric acid accounted for 28–66% of the total, with malic averaging 18–27% and unknown acids accounting for 12–52%. Gortner (1963) and Singleton and Gortner (1965), in

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