Table IV. Effect of Crystalline Vitamin B_{12} Supplementation on Liver Vitamin B_{12} Assay Activity of Baby Pigs Fed Experimental Rations

(Experiments II and III)

Expt. No.	Pig No.	Age, Days	Type of Ration	Vitamin B_{12} Injected, γ	Liver Activity, $m\gamma$ B_{12}/G.
II	5	56	Supplemented cow's milk	0	97
	8	56	Alpha-Protein ration	165	150
	9	56	Alpha-Protein ration	0	21
III	10	42	Washed Alpha-Protein ration	0	15
	11	42	Washed Alpha-Protein ration	210	250
	14	37	Drackett Protein ration	0	16

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VEGETABLE COMPONENTS

Some Carbohydrate Components of Tomato

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The carbohydrate components of the tomato were separated into free sugars and polysaccharides. The free sugars, representing more than 60% of the solids, were D-fructose, D-glucose, sucrose, and a ketoheptose. The polysaccharides, an araban-galactan mixture, a xylan-rich fraction, pectin, and α-cellulose, were present in comparable amounts.

IMPROVED PROCEDURES for the removal of more and more water from the tomato are constantly being investigated, because more economical methods are needed for the transportation and storage of tomato solids. The production of tomato pastes of high solids content and the preparation of dried tomato flakes or powders have led to problems in processing and storage stability. More detailed information on the composition of the tomato should aid in solving some of these problems. The results of this preliminary study of some of the carbohydrate components of the tomato may contribute such needed information.

Separation of Free Sugars From Polysaccharides

Table-ripe tomatoes were scalded in boiling water to facilitate removal of the skin. The peeled tomatoes were disintegrated in an electric blender. The resulting slurry was poured through a sieve to remove the seeds and then immediately added to sufficient boiling ethyl alcohol to give a concentration of 80% ethyl alcohol. The tomato pulp was extracted with copious amounts of hot 80% ethyl alcohol to remove the last traces of free sugars (*17*). The pulp containing the insoluble polysaccharides was washed with absolute ethyl alcohol and anhydrous ethyl ether to remove lipides and pigments and then air-dried.

Free Sugars

Eighty-one per cent of the solids in the peeled, seeded tomato pulp was found in the 80% ethyl alcohol extract. The total and reducing sugars were determined (*2*) and found to be 63.2 and 59.7%, respectively, of the tomato solids. Examination by paper chromatography (*16*) of the free sugars in this extract revealed substances indistinguishable from D-fructose, D-glucose, sucrose, and a ketoheptose. A sufficient quantity

of the extract was chromatographed for the determination of D-fructose and D-glucose (*15*) and their ratio was found to be 1.2 to 1.0. The remainder of the 17.8% of ethyl alcohol–soluble solids consisted of 6.2% protein (N × 6.25) and 11.6% of unidentified constituents that would contain, among other substances, ash and organic acids.

Polysaccharides

The 19% of tomato solids insoluble in 80% ethyl alcohol were presumed to be primarily polysaccharides and proteins (or nitrogen-containing compounds); this material is hereinafter referred to as the polysaccharides. This material was divided into various fractions by the use of solvents and enzyme preparations.

The polysaccharides contained 17.2% protein (N × 6.25), portions of which appeared in all fractions except one (Table I).

A sample of the polysaccharides was treated with saliva (*8*) and the extract therefrom was concentrated and examined by paper chromatography. There was no evidence of glucose or maltose on the chromatograms, indicating the absence of any tangible amount of starch.

To take advantage of the difference in solubility of arabans-galactans and pectin in alcohol-water mixtures, the polysaccharides were exhaustively leached with 50% ethyl alcohol at room temperature. The alcohol was removed from an aliquot of this filtered extract and the solution was made 1.0*N* with hydrochloric acid. The acidic solution was sealed in a glass tube and immersed in a boiling-water bath for 2 hours. The hydrolyzate was examined for sugars by paper chromatography. Substances indistinguishable from L-arabinose and D-galactose were found on the chromatograms. On other chromatograms of the same extract, prepared for the examination of uronic acids (*11*), a substance was found in juxtaposition with the lactone of D-glucuronic acid. The fraction soluble in 50% ethyl alcohol appears to be an araban-galactan mixture which includes glucuronic acid (Table I).

The material insoluble in 50% ethyl alcohol was air-dried and treated with a filtered solution of Pectinol 100D (*10*). Pectinol has been shown to contain enzymes capable of hydrolyzing maltose, sucrose, starch, inulin, xylan, protein, and modified celluose, in addition to pectin (*3, 4, 7, 13*). The filtered extract was allowed to stand 24 hours at room temperature to complete the conversion of pectin to galacturonic and oligogalacturonic acids. Uronic acids were determined by the carbazole method (*9*) and the pectic substances (anhydrouronic acid × 1.25) were estimated to be 22% of the polysaccharides. A portion of the extract was passed through columns of ion exchange resins Amberlite IR-120 and Duolite A-4, respectively. The effluent was concentrated and hydrolyzed with 1.0*N* hydrochloric acid as described above. Paper chromatography of the hydrolyzate revealed L-arabinose and D-galactose in quantity plus very small amounts of D-glucose and D-xylose.

The insoluble residue from the Pectinol-treated mixture was suspended in 0.06*N* hydrochloric acid, and crystalline pepsin was added in large excess to hydrolyze the remainder of the protein. After 3 hours of agitation at room temperature the suspended solids were removed by filtration. Protein equivalent to 9.1% of the polysaccharides was removed by this procedure. A portion of the filtrate was deionized, hydrolyzed, and chromatographed as described above. Substances indistinguishable from L-arabinose and D-xylose were found on the paper chromatogram. The arabinose was present in trace amount only (Table I).

The residue from the pepsin-treated mixture was suspended in 17.5% sodium hydroxide and stirred gently under nitrogen (*12, 19*) for 24 hours at room temperature. The undissolved material, α-cellulose, was removed by filtration through a sintered-glass crucible. Acid hydrolysis of the α-cellulose fraction produced D-glucose with traces of D-xylose and D-mannose (*1, 14, 19*) on the paper chromatogram (Table I).

The 17.5% sodium hydroxide filtrate was acidified with 50% acetic acid until a precipitate formed (*14, 18, 19*), at pH 5.0, and then 5 volumes of absolute alcohol were added. The precipitate was allowed to settle for 24 hours. The bulk of the liquid was removed by decantation and the precipitate was collected on a sintered-glass filter and thoroughly washed with 80% ethyl alcohol. The precipitated xylan was acid-hydrolyzed like the previous fractions. Paper chromatograms of the hydrolyzate showed D-xylose, D-glucose, and much smaller amounts of D-galactose and D-mannose (Table I) (*14*).

Table I. Carbohydrate Constituents of Tomato

Tomato. Peeled, seeded, and comminuted
Pulp extracted exhaustively with 80% ethyl alcohol
  Filtrate. 81% of tomato solids, composed of 59.7% fructose and glucose, 3.5% sucrose, 6.2% protein, and 11.6% of unidentified material which would include ash, organic acids, and other minor constituents
  Residue. 19% of tomato solids (polysaccharides)
Extracted exhaustively with 50% ethyl alcohol
  Filtrate. 12% of polysaccharides composed of substances hydrolyzed to L-arabinose, D-galactose, and D-glucuronic acid
  Residue. 88% of polysaccharides
Extracted with solution of Pectinol 100D
  Filtrate. 36% of polysaccharides composed of 22% pectic substances, 5% protein, and 9% of substances hydrolyzed to L-arabinose and D-galactose with small amounts of D-xylose and D-glucose
  Residue. 52% of polysaccharides
Extracted with acidic solution of pepsin
  Filtrate. 14% of polysaccharides composed of 9% protein and 5% material hydrolyzed to D-xylose with a trace of L-arabinose
  Residue. 38% of polysaccharides
Extracted, under nitrogen, with 17.5% sodium hydroxide
  Filtrate. 21% of polysaccharides composed of 8% substances hydrolyzed to D-xylose and D-glucose with small amounts of D-galactose and D-mannose, and 3% protein (N × 6.25). It is not known whether 10% material remaining in solution in acetic acid–ethyl alcohol mixture is of same composition as xylan precipitate.
  Residue. 17% of polysaccharides composed of substances hydrolyzed to D-glucose with trace amounts of D-xylose and D-mannose. There still remained, in this final fraction, 0.5% protein.

Discussion

No scheme has been reported for fractionating the alcohol-insoluble solids (the polysaccharides) quantitatively into substances of a single molecular species (*14*). This is due to the occurrence, in a single plant material, of polymers composed of one, two, or more sugars and to the variation in the molecular size of each polymer. The result has been the overlapping of solubilities in the solvents that have been tested. Commercial enzyme preparations that react specifically with a single molecular species of the type herein studied are, so far as known, unavailable.

The "quantitative" data of this report (Table I) were obtained under empirical conditions which are not offered as a method of analysis. The conditions selected were considered to be the most applicable for use with paper chromatographic procedures.

The free sugars represented 63.2% of the solids in the peeled, seeded tomato pulp. Chromatographic data showed that the only free sugars present were D-fructose and D-glucose, in the ratio of 1.2 to 1.0, a small amount of sucrose, and a trace of ketoheptose.

The polysaccharides (the tomato pulp insoluble in 80% ethyl alcohol) accounted for 19% of the tomato solids. The composition of this fraction included 22% pectic substances, 17% α-cellulose, and 17% protein (N × 6.25). This fraction also included, as suggested by chromatographic evidence, 13 to 23% of material composed primarily of D-xylose and D-glucose (exclusive of any D-glucose hydrolyzed from the α-cellulose), and 21% araban-galactan material. Paper chromatograms of the acid hydrolyzates of the araban-galactan mixture consistently showed, in addition to L-

arabinose and D-galactose, a uronic acid pattern comparable to that of authentic glucuronic acid. Starch could not be found in any tangible amount in the polysaccharides.

Many of the polysaccharide materials isolated from the tomato pulp were strongly lyophilic. Substances having such properties and occurring in such quantities must contribute to the consistency or to the "body" of products prepared from the tomato.

Every 100 grams of tomato solids contain 60 grams of reducing sugars and about 3 grams of protein. These substances may produce off-color and undesirable flavor as a result of the browning reaction (*5*, *6*) and, therefore, may be regarded as sources of trouble in the preparation of tomato concentrates.

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FLAVOR EFFECTS

Possible Relationship Between the Ionic Species Of Glutamate and Flavor

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The unique flavor effect obtained when monosodium glutamate is added to foods has led to its wide use in the food industry. The fact that the effect is most pronounced between pH 5.0 and 6.5 suggests that only one of the ionic species of glutamate may be responsible for its flavor properties. A study of the equilibrium of the ionic forms of glutamate as a function of pH has shown that one ionic form of glutamate is predominantly present in the pH range 4.5 to 7.0. Comparison between the calculated percentage of glutamate present in this ionic form and the intensity of the glutamate flavor is in good agreement with experimental data from the literature. The ionic theory explains the noneffectiveness of glutamate in acid foods as due to the low equilibrium concentrations of the flavor-active ionic form at pH values below 4.0. A method is presented for estimating total glutamate concentration for desired flavor effect above this lower pH limit.

TO THE MONOSODIUM SALT OF GLUTAMIC ACID is attributed a unique characteristic known as a "glutamic effect" or "glutability." This effect is manifested by a marked persistency of taste sensation, a stimulation of taste receptors, and what has been described as a "tingling feeling" or a "feeling of satisfaction" (*3*). This flavor-enhancing property has led to wide use of monosodium glutamate in the food industry, but it does not appear to be universally applicable to all foods—for example, fruits, fruit juices, sweet baked goods, some dairy products and cooked cereals, and some products containing relatively large percentages of fat. The most useful level seems to lie between 0.1 and 1% and generally between 0.1 and 0.3% of the total weight of the finished product (*5*).

The foods in which the flavor effect seems greatest appear to lie in the pH range of about 5.0 to 6.5. This so-called "pH effect" has led to the speculation that only one of the ionic forms of