THE CHEMISTRY

of

PLANTS

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PREFACE

An attempt has been made in this book to discuss the chemistry of plants without encroaching upon the field of plant physiology. Sometimes this is difficult because in describing chemical processes which occur in living plants it is often necessary to describe chemical reactions which a physiologist will also draw upon to explain fundamental processes. It is only natural that there will be a certain amount of overlapping in specialized sciences, since classifications are man-made. When there appears to be such an overlapping in this text it will be observed that the *chemistry* rather than the physiology is stressed. Thus, not respiration but the *chemistry* of respiration is discussed. The same is true of photosynthesis and other plant processes.

Material in this book has been extended to include topics not ordinarily covered in plant chemistry texts. For example, gums and mucilages are treated more or less extensively in the chapter on carbohydrates, and essential oils are discussed in the chapter on lipides. Separate chapters are devoted to glycosides and alkaloids. It is felt that these extensions are necessary if the text is to become a satisfactory reference book for specialists in other fields who require information on the chemistry of plants. It is believed that this book will be useful to medical students, research workers in medicine, agricultural chemists, and other specialists in both agriculture and the agricultural industries.

The writer of a textbook is a mere compiler. He cannot hope to have made original contributions to many of the areas included in a general text. He draws upon the contributions of many specialists. It is not possible to give credit to every one to whom it is due. An attempt has been made to do so where

possible. Specific mention has been made of authors and publishers who have permitted the use of illustrative material such as tables and figures. When not stated otherwise, the structural formulas for essential oils and alkaloids were adapted from illustrations in Pratt and Youngkin's "Pharmacognosy," published by J. B. Lippincott Company.

The writer is indebted to a number of friends and colleagues who have either offered valuable suggestions in the preparation of this book or who have read and criticized certain chapters. Chief among the former group is Dr. I. N. Kugelmass of New York City, who originally stressed the need for this book. Dr. Richard H. McCoy, University of Pittsburgh, has kindly read the first three chapters, and Dr. Jerome W. Rosenberg, University of Pittsburgh, has looked over Chapter 4. The chapter on Enzymes has been criticized by Dr. A. E. Axelrod, the chapters on Glycosides and Alkaloids by Dr. Edward P. Claus, and Chapter 12 by Dr. Richard T. Hartman, all of the University of Pittsburgh. Suggestions and criticisms on other chapters have been offered as follows: Dr. Aubrey W. Naylor, Duke University, Chapter 6; Dr. John W. Mitchell, U. S. Department of Agriculture, Chapter 7; Dr. Harold A. Wooster, Jr., Air Force Office of Scientific Research, the chapter on Vitamins; Dr. Paul Smith, U. S. Department of Agriculture, Chapter 11.

Typing and retyping of the manuscript has been done by Miss Geraldine Janciar, Mrs. G. S. Porter, Miss Mary Louise Corso, and Miss Sandra English, Department of Biological Sciences, University of Pittsburgh. Thanks are due to Daniel G. Bardarik, Jake Burkett, and Philip C. Hetrick for assistance with illustrations and to Cecelia Shelby and members of the writer's immediate family for assistance with proofreading.

Pittsburgh, Pa.   ERSTON V. MILLER
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1. CARBOHYDRATES

Carbohydrates were so named because they consist of carbon, hydrogen, and oxygen, the last two elements being combined in the same proportion in which they occur in water. They are not "hydrated carbons," however, and there are other organic compounds with the same type formula, $(CH_2O)_n$, but the term is still generally accepted. Carbohydrates in plants may be classified according to both function and chemical characteristics. Functionally they may serve either as constituents of the skeletal framework of plant cells or as sources of reserve energy, taking part in oxidative, hydrolytic, and synthetic mechanisms. The chemical classification will be employed here although the functional aspect will be brought out in discussing the distribution of specific carbohydrates. From the chemical standpoint carbohydrates may be classified into three groups: monosaccharides or simple sugars, oligosaccharides or compound sugars, and polysaccharides.

Sugars

The simplest form of carbohydrate is represented by the sugars. They are white, crystalline compounds, soluble in water and slightly soluble in alcohol, and are generally characterized by sweetness to the taste. Monosaccharides derive their individual names from the number of carbon atoms in the molecule. Thus we might have a biose, triose, tetrose, pentose, hexose, heptose, octose, nonose, and decose in this subgroup. In the plant, however, monosaccharides are represented primarily by pentoses and hexoses, although others, such as trioses and heptoses, may occur either in slight quantities or as transitory products.

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TABLE 1. A CLASSIFICATION OF THE PRINCIPAL CARBOHYDRATES*

I. Monosaccharides or simple sugars	1. Bioses 2. Trioses 3. Tetroses 4. Pentoses		Aldoses	1. Xylose 2. Arabinose 3. Ribose
			Ketoses	
	5. Hexoses		Aldoses	1. Glucose 2. Mannose 3. Galactose
			Ketoses	1. Fructose 2. Sorbose
	6. Heptoses 7. Octoses 8. Nonoses 9. Decoses			

D- and L- forms

II. Oligosaccharides or compound sugars	1. Disaccharides	1. Sucrose 2. Maltose 3. Gentiobiose 4. Trehalose 5. Melibiose 6. Cellobiose 7. Lactose
	2. Trisaccharides	1. Raffinose 2. Gentianose 3. Melezitose
	3. Tetrasaccharides	Stachyose

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III. Polysaccharides	1. Pentosans		1. Araban 2. Xylan
	2. Hexosans	1. Glucosans	1. Starch 2. Glycogen 3. Cellulose 4. Lichenin
		2. Fructosans	Inulin
		3. Mannans	
		4. Galactans	
	3. Pectic compounds		1. Pectic acid 2. Pectin 3. Protopectin
	4. Gums		
	5. Mucilages		
	6. Amino-hexosans		Chitin

Pentoses ($C_5H_{10}O_5$). Pentose sugars, though widely distributed in plants, do not occur in very large quantities. They are usually present as their condensation products or *pentosans*. In the pure form they rarely constitute more than one per cent of the dry weight of plants. Hydrolyses of such polysaccharides as gums, mucilages, and pectins yield pentoses. Boiling pentose sugars with 10 per cent hydrochloric or sulfuric acid produces furfuraldehyde. This distinguishes them from hexoses which yield furfuraldehyde only by treatment with concentrated mineral acids.

Following are some of the five carbon sugars which have been identified in plants. *Xylose* can be prepared from wood, straw, or bran. *Arabinose* is a constituent of gum arabic, cherry gum, etc. *Rhamnose* is obtained by hydrolysis of the water-soluble pigment, quercitrin, which occurs in oaks. *Ribose* is a constituent of some nucleic acids and of riboflavin, the latter being one of the B vitamins. The presence of a compound in small amounts is not necessarily an indication that it is not physiologically important. For instance, nucleic acid and riboflavin (which have just been mentioned as containing pentose sugars) play an important role in essential functions such as respiration and reproduction. *Desoxyribose* is another pentose sugar with functions similar to those of ribose, being a constituent of the nucleic acids of nuclei. D-Ribose is found in the nucleic acids of cytoplasm. Viruses (which are now known to consist of nucleoproteins) contain ribose nucleic acids. (See Chapter 2.)

Hexoses ($C_6H_{12}O_6$). *Glucose* and *fructose* are the six-carbon sugars commonly found in plants. Glucose, which is also known as dextrose, corn sugar, grape sugar, and blood sugar, is an aldehyde. It is dextrorotatory. Fructose is a ketone. This hexose is also called levulose or fruit sugar. Levulose is levorotatory. The prefix D or L is used on organic compounds to indicate that they are dextro- or levorotatory, respectively. Sugars, however, constitute an exception to this rule because the D and L in this case merely indicate a structural relationship to D- and L-glyceric aldehyde.
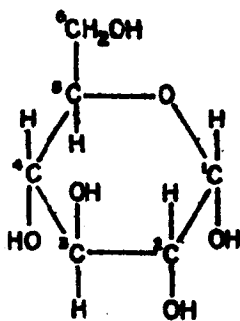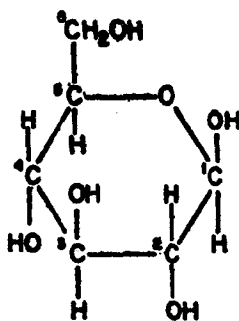
alpha-D-*Glucose*   beta-D-*Glucose*

Mannose and *galactose* are aldohexoses. They are found in plants only as traces. They occur more abundantly as condensation products, the mannosans, and galactans. The sugars are released from these condensation products by boiling with acids. Galactose has been observed to crystallize on the surface of berries of Virginia creeper (*Parthenocissus*) after the fruits have been frozen and has also been reported to form in sugar beets under abnormal conditions.

Plants may contain several sugar alcohols which are considered to be reduction products of hexoses. D-*Sorbitol*, a reduction product of D-glucose, is found in many plants of the Rosaceae, such as apple, peach, apricot, and cherry, and of Sorbus. Sorbitol may be reoxidized by certain microorganisms to L-sorbose, a ketose sugar. Other examples of these sugar alcohols are *mannitol*, a reduction product of D-mannose and D-fructose, and *dulcitol*, which is formed from galactose. Mannitol is of widespread occurrence in higher plants whereas dulcitol is of only rare occurrence, being present in the red algae and the higher plants *Melampyrum nemorosum* and *Euonymus atropurpureus*.

Disaccharides. Disaccharides are formed by the union of two hexose molecules. The two hexoses may be identical or each may be different. The most common disaccharide in plants is *sucrose*, which consists of a molecule each of glucose and fructose. Quantitatively speaking, the bulk of the sugars in plants consists of glucose, fructose, and sucrose. Plants like sugar cane and sugar beets, having been bred for high sucrose
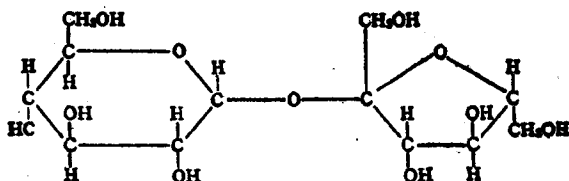
Sucrose

content, supply most of the sucrose of commerce today. The sugar maple tree and certain sorghums likewise contain large quantities of sucrose.

Two other interesting disaccharides are *maltose* and *cellobiose*. Maltose is an intermediate product in the digestion of starch and cellobiose is produced by enzymatic degradation of cellulose. Maltose is composed of two molecules of alpha-D-glucose whereas cellobiose contains two molecules of beta-D-glucose. *Trehalose* is a disaccharide which is found in fungi.

Other Sugars. Trisaccharides ($C_{18}H_{32}O_{16}$) and tetrasaccharides ($C_{24}H_{42}O_{21}$) are not widely distributed in plants. *Raffinose*, a trisaccharide, is found in cotton seeds and sugar beets in appreciable quantities and to a lesser extent in other plants. Upon complete hydrolysis this sugar yields D-galactose, D-glucose, and D-fructose. Another trisaccharide, *melezitose*, occurs in the European larch, Douglas fir, scrub pine, and other conifers. Two molecules of glucose and one of fructose result from complete hydrolysis of melezitose. The trisaccharide *gentianose* is found in the roots of *Gentiana lutea*, the yellow gentian. Complete hydrolysis yields one molecule of fructose and two of glucose.

Stachyose ($C_{24}H_{42}O_{21}$) is the only tetrasaccharide known to exist in plants, having been isolated from the hedge nettle (*Stachys tubifera*). It yields upon hydrolysis, one molecule each of glucose and fructose and two of galactose.

Di-, tri-, and tetrasaccharides are customarily grouped under the heading of oligosaccharides.

Properties of Sugars. Sugars may be classed as *reducing* or *nonreducing*. A reducing sugar contains either an aldehyde (—CHO) or a ketone (C=O) radical in its molecule. Nonreduc-

ing sugars are compound sugars in which the aldehydic or ketonic groups have lost their usual reactivity because of the manner in which the component monosaccharides are linked. All the monosaccharides and some of the more complex sugars are reducing sugars. The aldehydic or ketonic groups are readily oxidized; therefore they reduce oxidizing agents such as Fehling's or Benedict's solutions. When one of these solutions is heated with a reducing sugar, curpic hydroxide is converted to cuprous oxide, a red precipitate. The quantity of reduced copper bears a definite relationship to the amount of reducing sugar present and the reaction constitutes the basis of most quantitative methods for sugar analysis.
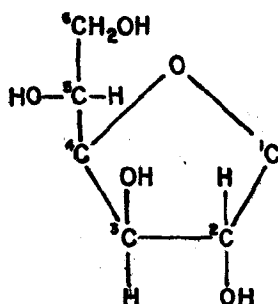
For the sake of simplicity, formulas for monosaccharides are usually written as straight carbon chains. There is good evidence, however, that these sugars possess a ring structure. Glucose and other hexoses may exist in the form of a pyranose ring or a furanose ring, the former being the more stable form. In the pyranose ring carbon atoms 1 and 5 are linked by an oxygen atom whereas in the furanose form the oxygen linkage is between carbon atoms 1 and 4.

Polysaccharides

Sugars in excess of the plant's immediate requirement for metabolism are converted into polysaccharides. These are complex carbohydrates which are condensation products or close derivatives of the monosaccharides. They are characterized by
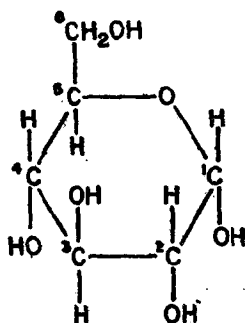
D-*Glucose (pyranose ring)*   D-*Glucose (furanose ring)*

high molecular weight. Condensation products of pentose sugars are called *pentosans*; those of hexoses, *hexosans*. In addition to the pentosans and hexosans, which are built of one kind of sugar, there are others of more complex nature which contain two or more different kinds of sugars or related compounds. Polysaccharides differ from the sugars in lacking sweetness and in not being soluble in water although many of them form hydrophilic colloids.

Pentosans ($C_5H_8O_4$). Pentosans commonly found in plants are the arabans and xylans, being condensation products of arabinose and xylose, respectively. Pentosans are found mainly in the cell walls of plants where they serve principally as reserve foods. Arabans are found in the natural gums of cherry, peach, and plums. Xylans occur in the shells of apricot seeds and in wood, straw, and bran. Pentosans are capable of imbibing large quantities of water. They surpass hexosans in this regard. The water-holding power of drought-enduring plants, such as cacti, is traceable to the presence of pentosans which are constituents of mucilaginous materials found in these plants. After the more readily utilizable materials like starch and dextrins have been exhausted, pentosans may then be drawn upon for reserve foods in plants. Many wood-rotting fungi, like *Xylaria* sp., utilize pentosans as a source of energy.

Hexosans ($C_6H_{10}O_5$). Polysaccharides of this type are similar to pentosans in consisting of condensations of simple sugars but differ in that the sugar molecule of the chain is hexose rather than pentose.

The hexosan *cellulose* is the fundamental constituent of the plant cell wall. When the primary wall of the plant cell is first formed it consists largely of cellulose. As the wall matures the cellulose layers may become impregnated with substances like lignin, cutin, or suberin. The presence of cellulose in the cell wall is almost a universal characteristic of plants. The only known exceptions are the slime molds (myxomycetes) in the ameboid stage (when cellulose is lacking) and the diatoms, in which cellulose is replaced by silica.

Cellulose is said to be the most abundant organic compound

on the earth. It is the basis of all wood fibers. Cotton and linen are nearly pure cellulose. Cellulose may be hydrolyzed to glucose by treatment with concentrated sulfuric or hydrochloric acids. In dilute solutions of sulfuric acid cellulose merely swells and becomes *hydrocellulose*. Similar results are produced by treatment with 15 per cent sodium hydroxide. Cellulose is insoluble in water but may be dissolved by Schweitzer's reagent (ammoniacal copper hydroxide),

Because of its general insolubility and inert characteristics, cellulose serves as structural material in the plant rather than as a reserve food. Neither does man utilize this carbohydrate for food. Lower organisms, such as bacteria and fungi, digest plant residues so that they may be returned to the soil. Termites are capable of digesting cellulose but actually the process is carried on by a protozoan in the intestinal tract rather than by the digestive juices of the termite. A few of the higher animals which also digest cellulose likewise are dependent upon microorganisms in the digestive system to accomplish the actual breakdown of cellulose.

The molecule of cellulose consists of a long chain of beta-D-glucose residues. There may be a thousand or more of these glucose residues linked together by oxygen bridges. Almost nothing is known regarding the mechanism by which cellulose is synthesized in plants.

Hemicelluloses

The term *hemicellulose* is applied to certain polysaccharides in the cell wall of plants which, though cell-wall constituents, may also serve as reserve foods. They have also been referred to as hemi-reserve materials. Older methods for determining

Cellulose
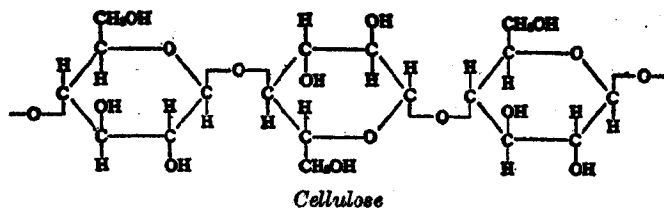
these compounds, such as hydrolysis by dilute mineral acids, would naturally include carbohydrates which have been listed in other categories, such as pentosans, galactans, mannans, and pectic substances. Products of hydrolysis of hemicelluloses include D-glucose, D-galacturonic acid, D-xylose, D-galactose, D-glucuronic acid, L-arabinose, and D-mannose. Hemicelluloses occur in seed coats, nut shells, and woody tissues and are usually associated with lignins. The cell walls of the woody tissues of some trees contain hemicelluloses. These polysaccharides are digested in the spring, supplying energy for the new growth of the stems. Likewise hemicelluloses stored in many seeds serve as sources of food for the young seedlings during germination. The seed of the South American ivory nut palm (*Phytelephas macrocarpa*) is an example of this type of seed. Hydrolysis of the nuts yields dextrose, levulose, mannose, and pentoses. The nuts have yielded as much 40 per cent of the dry weight in mannose. Seeds of the date palm are also of this type, the embryo being embedded in a horny endosperm of hemicellulose.

E. M. Mitchell studied the hemicelluloses in ten different seeds and found considerable variation in chemical characteristics. Other investigators have reported the following sugars among the hydrolytic products of those hemicelluloses: arabinose, xylose, glucose, fructose, galactose, and mannose.

Although the complete chemistry of the hemicelluloses is not fully understood we do know that many of them are polyuronides. They are composed of glycosidic chains containing both uronic acid and pentose. Uronic acid is the general term applied to a hexose which has been oxidized without being dismembered. Thus the oxidation of glucose at carbon 6 becomes D-glucuronic acid. In like manner, galactose may be oxidized to D-galacturonic acid. Polyuronide hemicelluloses of one type contain glucuronic acid and xylose. Another type contains galacturonic acid and arabinose.
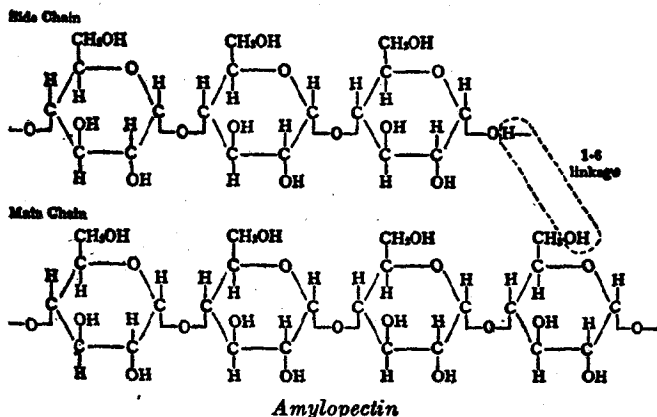
Starch

Starch differs from cellulose both in function and structure. Unlike cellulose it is an important food for both plants and

animals. Soon after photosynthesis begins, starch grains appear in the chloroplasts and they continue to accumulate during the daylight hours. As a food reserve, starch is stored in seeds, roots, tubers, and other storage organs as well as in the stems of plants. Since starch is insoluble in water it is first hydrolyzed to sugar before being translocated to nonchlorophyllous parts of plants for storage. The shape of starch grains is constant within species; thus the source of the grain can be determined by microscopical examination.

Starch consists of two components: amylose and amylopectin. Both are condensation products of alpha-D-glucose. In amylose, glucose molecules in the pyranose form are linked between carbon atom one of one molecule and carbon atom four of the adjacent molecule. Amylopectin molecules have a multiple-branched rather than a straight chain. Straight chains in amylopectin are constructed similar to those in amylose but there are also many side chains in which carbon atom six of certain glucose residues is linked with carbon atom one of the other glucose molecules.

Hydrolysis of starch to simpler substances is brought about by *amylases*. Alpha-amylase converts starch to dextrins; beta-amylase hydrolyzes the amylose portion of starch to maltose and the amylopectin fraction partly to maltose and partly to dextrins. There are apparently other enzymes which carry the

Amylopectin

reactions on to maltose. The enzyme *maltase* converts maltose to glucose. *Diastase*, which consists of a mixture of amylases and maltase, carries the hydrolysis of starch to completion, i.e., to glucose.

Synthesis of Starch. It has been mentioned previously that soon after photosynthesis begins, starch grains begin appearing in the chloroplasts. This does not mean, however, that starch synthesis is a part of the photosynthetic process, for starch grains are formed in leucoplasts and in nonchlorophyllous organs of plants like roots and tubers. Furthermore, laboratory experiments have demonstrated that if etiolated leaves are floated on sugar solutions in the dark they are capable of synthesizing starch.

The actual mechanism of starch synthesis consists of a phosphorylating process. The phosphoric ester, glucose-1-phosphate, appears to be the starting point in this process. Catalyzed by the enzyme alpha-glucosan phosphorylase, phosphoric acid is removed from one molecule of glucose-1-phosphate and the residue is linked to another molecule of glucose-1-phosphate through an oxygen bridge. The process is repeated with another molecule of glucose-1-phosphate until a long chain of *glucose residues* becomes a molecule of starch.

There are some plants which do not synthesize starch in the leaves although photosynthesis occurs in these organs just as in those of other green plants. Many species of the Liliaceae, Amaryllidaceae, Gentianaceae, Compositae, and Umbelliferae show this characteristic. H. C. Eyster has studied this phenomenon in the onion plant and has reported the presence of an inhibitor of starch synthesis in the leaves.

Glycogen, sometimes called animal starch, is found in some species of bacteria and fungi but rarely in higher plants. It is the storage carbohydrate of animal tissues, being present in liver and muscle tissues. A carbohydrate resembling glycogen has been obtained from Golden Bantam sweet corn. Structurally glycogen consists of branched molecules, the glucose residues being linked through the 1-4 and 1-6 carbon atoms.

All of the hexosans thus far mentioned have been glucosans.

The only well-known fructosan is *inulin*. This compound accumulates in storage organs of Jerusalem artichoke, chickory, dahlia, dandelion, salsify, and goldenrod. Inulin is seldom if ever found in the aerial parts of plants.

General Consideration of Sugar

Ripening in fruits is usually accompanied by a decrease in organic acids and in increase in sugars. Best dessert quality occurs when the ratio of sugar to acid is such as to impart a pleasantly tart taste to the fruit. In one type of fruit, like apples, pears, and bananas, the fruits are harvested in the "market ripe" or "mature green" stage of maturity, at which time they contain relatively large quantities of starch. During ripening the starch is converted to sugars. Citrus fruits do not contain a starch reserve so that the sugar which accumulates during ripening is directly dependent upon the photosynthetic process. Fruits of this type must be ripened on the tree. The lemon differs from most other fruits in that ripening is accompanied by increase in acids rather than an increase in sugar.

Vegetables like peas, beans, and corn are frequently consumed in the "immature-seed" stage, at which time they contain a maximum amount of sugar and the ratio of starch to sugar is such as to produce desirable texture and succulence. The sugar is converted to polysaccharides as the seeds mature. At this stage they are not quite as desirable for food, though they are entirely satisfactory for planting.

Glucose, fructose, and sucrose make up the bulk of sugars found in plants. The ratio of reducing sugar to nonreducing sugar (mainly sucrose) varies with the type of plant or plant organ, or maturity of the plant or plant part. Field corn leaves at 3:00 P.M., for example, have been shown to contain more than four times as much sucrose as reducing sugar. Mature Pineapple oranges in December contained roughly equal quantities of both types of sugar, whereas ripe Jonathan apples at the time of commercial harvest have been found to contain 6.41 per cent reducing sugar and 2.89 per cent sucrose. Fresh peas and beans, on the other hand, have been reported to con-