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Sugar Substitutes and Enhancers

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SUGAR SUBSTITUTES AND ENHANCERS

Roger Daniels

NOYES DATA CORPORATION
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FOREWORD

The detailed, descriptive information in this book is based on U.S. patents since 1960 relating to the manufacture and application of sugar substitutes and enhancers. Where it was necessary to round out the complete technological picture, some earlier but very relevant patents were included, as were some British patents of importance.

This book serves a double purpose in that it supplies detailed technical information and can be used as a guide to the U.S. patent literature in this field. By indicating all the information that is significant, and eliminating legal jargon and juristic phraseology, this book presents an advanced, commercially oriented review of sugar substitutes and enhancers.

The U.S. patent literature is the largest and most comprehensive collection of technical information in the world. There is more practical, commercial, timely process information assembled here than is available from any other source. The technical information obtained from a patent is extremely reliable and comprehensive; sufficient information must be included to avoid rejection for "insufficient disclosure."

The patent literature covers a substantial amount of information not available in the journal literature. The patent literature is a prime source of basic commercially useful information. This information is overlooked by those who rely primarily on the periodical journal literature. It is realized that there is a lag between a patent application on a new process development and the granting of a patent, but it is felt that this may roughly parallel or even anticipate the lag in putting that development into commercial practice.

Many of these patents are being utilized commercially. Whether used or not, they offer opportunities for technological transfer. Also, a major purpose of this book is to describe the number of technical possibilities available, which may open up profitable areas of research and development. One should have to go no further than this condensed information to establish a sound background before launching into research in this field.

Advanced composition and production methods developed by Noyes Data are employed to bring our new durably bound books to you in a minimum of time. Special techniques are used to close the gap between "manuscript" and "completed book." Industrial technology is progressing so rapidly that time-honored, conventional typesetting, binding and shipping methods are no longer suitable. We have bypassed the delays in the conventional book publishing cycle and provide the user with an effective and convenient means of reviewing up-to-date information in depth.

The Table of Contents is organized in such a way as to serve as a subject index. Other indexes by company, inventor and patent number help in providing easy access to the information contained in this book.

15 Reasons Why the U.S. Patent Office Literature Is Important to You—

(1) The U.S. patent literature is the largest and most comprehensive collection of technical information in the world. There is more practical commercial process information assembled here than is available from any other source.

(2) The technical information obtained from the patent literature is extremely comprehensive; sufficient information must be included to avoid rejection for "insufficient disclosure."

(3) The patent literature is a prime source of basic commercially utilizable information. This information is overlooked by those who rely primarily on the periodical journal literature.

(4) An important feature of the patent literature is that it can serve to avoid duplication of research and development.

(5) Patents, unlike periodical literature, are bound by definition to contain new information, data and ideas.

(6) It can serve as a source of new ideas in a different but related field, and may be outside the patent protection offered the original invention.

(7) Since claims are narrowly defined, much valuable information is included that may be outside the legal protection afforded by the claims.

(8) Patents discuss the difficulties associated with previous research, development or production techniques, and offer a specific method of overcoming problems. This gives clues to current process information that has not been published in periodicals or books.

(9) Can aid in process design by providing a selection of alternate techniques. A powerful research and engineering tool.

(10) Obtain licenses – many U.S. chemical patents have not been developed commercially.

(11) Patents provide an excellent starting point for the next investigator.

(12) Frequently, innovations derived from research are first disclosed in the patent literature, prior to coverage in the periodical literature.

(13) Patents offer a most valuable method of keeping abreast of latest technologies, serving an individual's own "current awareness" program.

(14) Copies of U.S. patents are easily obtained from the U.S. Patent Office at 50¢ a copy.

(15) It is a creative source of ideas for those with imagination.

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INTRODUCTION

Experts believe there are four basic tastes: sweet, salty, sour, and bitter. These tastes can be represented typically by sucrose, sodium chloride, tartaric acid and quinine sulfate. Sugars include mainly sucrose, dextrose, starch syrups and corn sugars. The latter are a family of tailor-made standardized starch or corn sugars resulting from the partial hydrolysis (called conversion) of starch followed by clarification and concentration to syrup density. Starch sugar or corn sugar is prepared by crystallization or drying of syrup concentrates. Refined corn sugar, dextrose or glucose are the names for the white, crystallized and pure sugars prepared by complete hydrolysis of starch.

The U.S. production of sugars is about 20 billion pounds per year, worth more than $1.5 billion. Sugars are used not only for sweetness but also for functional purposes, for example, flavor, body, texture, color development, and as a dispersant, fixative, bulking agent and other uses. In addition, there are many complexing reactions of sugars with proteins, fats, emulsifiers, and other food ingredients. In other words, sugar contributes to the acceptability of foods in many different ways.

A sweet taste is produced by many aliphatic hydroxyl compounds, particularly alcohols, glycols, sugars and sugar derivatives. It is theorized that the hydroxyl groups must be on adjacent carbon atoms in order to obtain the property of sweetness. The strength of the sensation sweet can not be measured quantitatively in absolute terms, but must be tested by subjective methods using human subjects. Since no absolute rating for sweetness is possible, only relative sweetness can be reported. Comparisons are usually made with sucrose as the standard.

Prior to 1970 saccharin and cyclamate were used in foods and beverages as non-nutritive sweeteners. On October 17, 1969 an order was issued by the Food and Drug Administration stating that cyclamate can no longer be generally recognized as safe and no longer can be used in foods (see Appendix).

Cyclamic acid and its salts were used in greater amounts than saccharin, on a weight basis, because they were approximately one-tenth as sweet as saccharin, and also they were used in combinations with saccharin in high ratios ranging from 1:5 to 1:12 saccharin to cyclamate. It was found that these combinations gave less aftertaste and less bitterness than when either sweetener was used alone.

Many processes are described in the patent literature on the use of cyclamate and its combinations with saccharin for use in foods and beverages. Because of the 1969 ban on the use of cyclamates no processes are included in this publication which give cyclamate as

the sweetener. However, in several instances processes and examples are given which were originally written for saccharin-cyclamate combinations, and in which the principles and approaches also apply if saccharin is used alone. In the formulations cited for these examples, the amounts of ingredients listed are approximations since they were based originally on the inclusion of cyclamates.

The production of artificial sweeteners prior to 1970 was over 10 million pounds per year with a value of $15 million. Presently, U.S. production of saccharin is 4.5 million pounds with substantial imports from Korea and Japan. The two leading U.S. producers are Monsanto and Sherwin-Williams.

The biggest single use of saccharin is in soft drink products accounting for 70% of the saccharin sold in the U.S. In 1965, 17% of soft drinks were dietetic type. In 1970, it was well over 35% and is still growing. The dietetic soft drink industry is reported to be a $500 million market.

Over the past ten years a great deal of work has been done to identify and develop other non-nutritive sweeteners as replacements for saccharin. None of these will be available for two to five years because of the extensive testing required by the Food and Drug Administration before they are allowed in foods and beverages. Screening programs for new natural materials with high sweetness intensity have been undertaken by the U.S. Department of Agriculture and other research groups. There has been considerable interest and activity in the use of natural materials to induce or expand the sweetness of natural sugars so that lower sugar levels can be used for lower caloric content. All these approaches are covered in this volume.

The reader is reminded to refer to the Appendix for important directives by the Food and Drug Administration on the status of cyclamates and the requirements on the use of saccharin and labeling of products containing saccharin and natural sweeteners.

On February 26, 1973, the Food and Drug Administration issued a statement that the National Academy of Sciences is reviewing the recent findings reported by the University of Wisconsin on animal feeding studies with saccharin. At the time of printing of this publication, there is considerable uncertainty on the future status of saccharin as a food additive. Due to these circumstances, the reader is advised to determine the official status of saccharin from reliable sources before this ingredient is used in a food product. In the event that saccharin is banned, the application of many of the principles covered in the processes contained in this book will be valid and useful for new or substitute artifical sweeteners and sweetening systems.

MIRACULIN - GLYCYRRHIZIN - ARTICHOKE SWEETENERS

Since the cyclamate ban there has been considerable interest in natural sweeteners with high sweetness intensities to replace ordinary sugars to reduce calories. In this chapter the three most important classes of natural materials are reviewed. These materials are extracted from natural products, and if used without chemical modifications they are acceptable by the Food and Drug Administration for use in food products.

MIRACULIN

Stable Solid Product

Synsepalum dulcificum Daniell, Sapotaceae is a plant indigenous to West Central Africa which bears a red ellipsoid fruit commonly known as "miracle fruit". The fruit has a palatable pulp and skin and contains a large seed. It is characterized by a pleasant taste and by the unique property, well-recognized for over 200 years, of modifying the sweet and sour tastes in an unusual manner. It has been found that a component in the fruit depresses the sour taste and accentuates the sweet taste of any normally sour food eaten within a short period after first contacting the tongue with the pulp of fresh miracle fruit, thus causing the normally sour food to taste pleasantly sweet. By exposing the taste receptors on the tongue to miracle fruit, any sour tasting food can be made to taste sweet without the addition of sugar or artificial sweeteners. For example, fresh lemon can be made to taste pleasantly sweet by first eating a miracle fruit berry. The taste-modifying principle in the miracle fruit berry known as miraculin binds itself to the taste receptors thus altering the sensory perception of the sour taste in foods eaten after the miracle fruit.

It has been determined that miraculin is a glycoprotein having a molecular weight of about 44,000. A wide variety of approaches have been explored in attempts to isolate the active component in miracle fruit for subsequent use as a taste-modifying material. These attempts have met with only limited success since the form of the product obtained by these methods is less effective than the natural fruit, and was found to be highly unstable at normal room temperatures under normal atmospheric conditions. This instability necessitated either very quick use after isolation or storage at very low temperatures.

The prior art has regarded miraculin as a very labile material accounting for the observed instability of concentrates maintained at normal room temperatures. While some degree of success has been attained in improving stability of the miraculin-containing material in the order of about a week or so, it has been found that its stability could not be achieved when maintained in powder form at room temperatures. Alternatively, the

miraculin-containing material was dissolved in specific solvents maintained at a specific pH. These solutions had to be refrigerated to be preserved, and even then this material was not as effective as the natural fruit.

The miraculin is present in the pulp and on the inner surface of the skin of the miracle fruit and in its natural environment is quickly deactivated especially when exposed to the air once the shin is broken at room temperatures. Furthermore, after the fruit has been picked, even prior to breaking the skin, the active material begins to degrade but at a slower rate than when the skin is broken. While the process by which degradation proceeds is not known exactly, it is now believed that certain enzymes and/or acids present in the fruit accelerate degradation in the presence of air at normal room temperatures, and apparently even at temperatures below the freezing point of water. It has been found that when the pulp of miracle fruit is frozen and subsequently lyophilized to form a granular or powder material, the product had to be refrozen in order to maintain the activity of the material that remained. Even when the pulp had been lyophilized, its effectiveness was not nearly as great, either on a weight basis or on a quality basis, as the active principle in the fresh fruit.

J.R. Fennell and R.J. Harvey; U.S. Patent 3,676,149; July 11, 1972; assigned to Meditron, Inc. described a process for extraction of miraculin and preparation of a stable product in powder form. A stable miraculin-rich composition is obtained by comminuting depitted ripe miracle fruit containing miraculin and then separating the vaporous and liquid components including acids and enzymatic components of the ripe fruit that degrade miraculin from the miraculin-rich material. The liquid and vaporous components are separated by dehydration and the enzymatic components are separated by any means that effects separation on the basis of density.

To minimize miraculin loss after picking, the whole fruit can be frozen to very low temperatures to await processing or the pulp and skin can be processed immediately after picking to obtain the concentrated miraculin. Comminution of the fruit serves to fracture the cell walls and thereby expose substantially all of the miraculin and facilitate subsequent processing. Dehydration can be effected in any convenient manner wherein low temperatures can be obtained including lyophilization, foam separation, spray drying or similar dehydration, processes and can proceed or follow the separation step based on density. It is preferred to separate the high density miraculin from the low density enzyme-rich material following dehydration because of the increased efficiencies obtained. If the miraculin is not separated from the material containing the enzyme, the product is unstable and will be degraded quickly at normal room conditions so that it loses its taste-modifying effect.

The miraculin-rich material, substantially free of the degrading enzyme and/or acids, has a substantially higher density than the material containing the enzyme. Therefore, the separation of the miraculin, that may contain some cellulosic material, from material containing the enzyme is effected by processes that separate materials on a density basis. To facilitate this separation, the mixed pulp and miraculin is preliminarily comminuted and screened to obtain uniformly small particle size.

The process is based upon the discovery that degradation of miraculin in the fruit is initiated immediately after the ripe fruit is picked, and that degradation of the active principle in its natural environment is accelerated by increased temperature and by contact with air. Thus, it is preferred to process the ripe miracle fruit as quickly as possible, at as low a temperature as possible, and in a nonoxidizing atmosphere, to obtain a high yield of miraculin. Preferably, the picked fruit is washed in water and then depitted at about 1°C. to 4°C. The fruit can be stored in a frozen state to await processing or can be processed immediately to obtain the active principle. When stored, temperatures of about -40°C. or less are employed to arrest degradation since it has been found that degradation of the active principle in the frozen fruit occurs even when stored at temperatures of about -15°C. Since it is difficult to remove the pit or seed from the frozen berry, it is preferred to depit the berry prior to frozen storage. The depitted berry, regardless of whether it

has been stored previously or whether it is processed directly after having been picked, is comminuted in a frozen state either alone or together with Dry Ice or ice formed from pyrogen-free distilled water. When the berry is processed immediately after having been picked, the pulp and skin obtained from the depitting step are directed into a container placed in a low temperature bath which itself may contain crushed Dry Ice. The pulp and skin are then comminuted at low temperatures such as by blending, grinding or ball-milling with ball-milling in a shell freezer being preferred.

In addition to the lyophilization dehydration process described above any other form of dehydration commonly used in food processing can be used as long as the temperature can be controlled and maintained low during dehydration. It is preferred to use Dry Ice in the container receiving the pulp from the depitting step and during the comminuting step. The frozen carbon dioxide serves two important functions; it provides low operating temperatures in the order of -40°C. or less as well as a CO_2-rich atmosphere known to preserve vegetable matter. Also, since Dry Ice sublimes, it is readily separated from the frozen pulp.

The pulp is comminuted until the average particle size of the mixture is about 600 microns or less. It is preferred to comminute the pulp to a particle size on the order of 100 to 125 microns to insure breaking of substantially all of the cell walls. Preferably, the mixture then is lyophilized under vacuum at a temperature of about -40°C. or less to remove liquid and vaporous components to include certain organic acids such as formic acid. Freeze drying is continued until there is no significant weight change in the material over about a four hour period. At this point in the process, the dried pulp contains less than about 5 weight percent moisture. To remove the remainder of the liquid and volatiles from the pulp by lyophilization would require an inordinately long period of time. Therefore, it is best to complete the dehydration in a desiccator at normal room temperatures. The small concentration of moisture in the pulp during desiccation will not cause significant degradation of miraculin during the final drying period. The miraculin then is separated from the enzyme-rich material on the basis of density.

The separation of the miraculin-rich material from the enzyme-rich material is based upon the fact that the active principle is considerably more dense than the enzyme-rich material in the order of about 10 times as dense. Thus, the mixture of inert and active material described above can be separated by any convenient density separation method including settling from a suspension of the mixture in liquid, the use of fluidizing bed technique, or through the use of cyclone type centrifuge. Some separation of miraculin-rich material from enzyme-rich material can be effected prior to dehydration by placing the comminuted pulp in settling pans at a temperature of 1° to 4°C. until the highest density material has settled in the bottom. The settling is complete in a short period of about 20 minutes with the miraculin-rich material forming the lowest layer which is then separated. The material then is frozen to below about 40°C. and dehydrated. The preferred separation method is conducted after dehydration and utilizes a cyclone type centrifuge for dry powder following dehydration described in the examples.

The miraculin-rich material has a density in excess of 1 g./cc while the enzyme-rich cellulosic material has a density of less than about 0.5 g./cc. Since the high density material is white and the low density material is brown the separate layers can be easily and quickly identified on the basis of color. To obtain the desired product stability, enzyme-rich material concentration should be as low as possible with removal in the order of about 95% having been found to be adequate.

The active principle obtained by this process is insoluble in water and only partially soluble in saliva. To be effective in suppressing sour taste and enhancing sweet and salt taste, the product must be applied to the sour taste receptors on the tongue. The miraculin-rich material can be applied conveniently as a powder or in a unit dosage form admixed with inert solids such as a tablet, capsule or gum, or coated on the unit dosage form or admixed with water or solvents for use as a liquid spray or the like. The active principle is retained on the tongue a sufficient period to contact essentially all of the sour taste receptors.

When employed in unit dosage form, as little as about 0.1 milligram of miraculin with a product of small particle size are required to obtaine the taste-modifying effect. Miraculin is employed in unit dosage forms in amounts of from 0.1 to about 50 milligrams, usually from 10 to 50 milligrams.

Example 1: Ripe miracle fruit berries are picked and washed at 1° to 4°C. in a water-ice bath in an insulated container. The fruit then is depitted at about 1° to 4°C. in a juicer comprising a perforated cylinder housing a rotating brush extending along the cylinder length the ends of which contact the inside cylinder wall. During rotation, the brushes tumble and press the berries against the perforated housing causing the juice and pulp material to pass through the holes and leaving the pits in the cylinder. The juice and pulp flow into containers that contain Dry Ice and are immersed in an alcohol-Dry Ice bath. Crushed Dry Ice (solid CO_2) is added directly to the fruit pulp obtained from the depitting step, and the mixture is thoroughly ground in a ball mill to a particle size of less than 150 microns (100 sieve size) while being maintained at a temperature of about -40° to -50°C.

The mixture then is placed in a freeze drying flask, placed in a shell freezer, and allowed to come to thermal equilibrium at a temperature of about -55°C. The material is then connected to a freeze-dryer vacuum system with a refrigerated condenser for condensing liquids and condensable vapors, where it remains until there is no significant weight change in the material over a four hour period. The material is then removed from the flask and placed in a desiccator cabinet in trays at room temperature for further drying or storage until the moisture content is substantially zero.

After the powder is thoroughly dry, it is placed in a temperature controlled milling machine, where the average particle size is reduced preferably to less than about 150 microns. The material is periodically screened and that retained by the 100 sieve size is returned to the milling machine until it can pass the 100 sieve size.

The fine powder is introduced into a pneumatic cyclone-type separator, whereby the dense miraculin is concentrated near the inside wall and the cellulosic material is concentrated closer to the center of the cyclone. The mixture to be separated is introduced into the top of the cyclone and caused to move in a circular path down the inside wall. The miraculin-rich material is separated from the lower density material by a baffle located at the interface of the miraculin and lower density material. The lower density material is recycled until substantially all the miraculin is separated. The concentrated miraculin can be recycled if necessary, to achieve any degree of separation from the lower density material. The miraculin powder obtained from the cyclone separator is room temperature stable even when stored in the open atmosphere for at least about 8 months and can then be used to produce unit dose forms including tablets or aqueous sprays.

Example 2: This example illustrates a typical miraculin formulation, a method for preparing chewable tablets therefrom and the results of tests on subjects ingesting the tablets. The formulation used to make the tablets is set forth in the following table.

Identification	Amount, milligrams
Lactose, direct tableting grade	248.3
Sorbitol, direct tableting grade	80.0
Flavoring	7.0
Coloring	0.7
Magnesium stearate	13.0
Per tablet	349.0

The following procedure was carried out at a temperature of 68° to 75°F. with relative humidity of less than 50% to prepare the tablets. The ingredients set forth in the table were mixed and blended with miraculin prepared as described in Example 1 at a concentration of 50 milligrams miraculin per tablet. The result of the mixture was screened to

pass through a 20 sieve size. The tablets were made by pressing the formulation in a Stokes Rotary Tablet Press (B2) using a standard 12/32 inch concave punch. The tablets had a hardness (Monsanto) of 3.0 to 3.5 kg. and weighed 399 milligrams.

The tablets were tested for their taste-modifying effects by a procedure that determined the apparent sweetness effected by a standard citric acid solution after ingesting the miracle fruit tablet and compared this sweetness to sugar solutions of varying concentration.

Each subject rinsed his mouth for one minute with distilled water. The miracle fruit tablet then was thoroughly chewed for one minute. Then the subject rinsed his mouth with distilled water for thirty seconds and waited two minutes. The subject then tasted a standard solution comprising citric acid (0.00926 M) which had a sourness equivalent of 0.01 M hydrochloric acid. After rinsing with the citric acid solution, the subject rinsed with distilled water for thirty seconds. After experiencing the sweetness of the citric acid solution, the subject was then required to compare the sweetness experienced with one of 11 standard sugar solutions.

The sugar solutions had varying concentrations as follows: 0.1000 M, 0.1175 M, 0.1379 M, 0.1620 M, 0.1993 M, 0.2236 M, 0.2626 M, 0.3083 M, 0.3622 M, 0.4256 M, and 0.5000 M. The subject then was asked to write down which, if any, standard solution most closely compared to the sweetness experienced with the citric acid after taking the miracle fruit tablet.

The biological assay procedure indicates that the tablets are effective in substantially increasing the sweetness of the normally sour citric acid solution. Usually the citric acid is comparable in sweetness to either the 0.2626 M or 0.3083 M sugar (sucrose) solution.

Although there were 200 different subjects tested, several having used the tablet in their daily diet, approximately 95% found the tablet to be effective in causing the citric acid to taste sweet, in sweetening other sour tasting foods and enhancing the flavor of almost all types of fruits and vegetables tasted. After six months of storage in a glass bottle at room condition with no particular care being taken in handling and storing of the tablets to maintain them dry, the tablets were still stable and effectively modified taste.

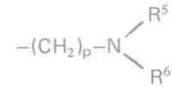
Solubilizing Method

In the process of *J.N. Brouwer, G.J. Henning and H. van der Wel; U.S. Patent 3,682,880; August 8, 1972; assigned to Lever Brothers Company* a solubilizing agent is used to treat the flesh, pulp, or seeded fresh or frozen fruit. Suitable solubilizing agents fall into two groups: (1) Compounds which dissolve the active factor in a form having a molecular weight (as determined by gel filtration) of above about 50,000, generally above 200,000, and apparently representing a bound form of the active factor. (2) Compounds which dissolve the active factor in a form having a molecular weight of below about 50,000 and from which the pure active factor is preferably prepared.

The first group includes: (a) Tannin-binding substances, e.g., polymers such as polyvinylpyrrolidone, sorbitan mono-oleate dipolyethyleneglycol ether, and polyethyleneglycols with mean molecular weights above about 200, proteins such as gelatin, casein, and albumins, peptones, caffeine, and salts such as aluminum sulfate; (b) Hydroxycarboxylic acids such as ascorbic acid and tartaric acid, and acylated neuraminic acids such as N-acetylneuraminic acid and N-glycolylneuraminic acid.

When solubilizing agents of the second group include: (a) Protamines, such as salmine; (b) Polypeptides prepared from basic amino acids, such as polyarginine; (c) Polyamines of the general formula:

in which n is an integer from 1 to 8, R^1, R^2, R^3, and R^4, may represent hydrogen, alkyl groups with not more than four carbon atoms or



in which p is an integer from 1 to 8 and R^5 and R^6 are hydrogen or alkyl groups with not more than four carbon atoms, provided that where R^1 to R^4 all denote hydrogen, n is greater than 1.

When solubilizing agents of the second group are used, the pH of the homogenate is adjusted to 3 or above and, in fact, when protamines are used preferably to values between 6 and 10 and when polyamines are used preferably between 6 and 8. The processes are carried out at low temperatures of up to 30°C., preferably at a maximum temperature of 5°C.

The constituents remaining insoluble after homogenization are removed by centrifuging, preferably at 10,000 to 30,000 *g* and/or by filtration through glass. (Since the active factor is absorbed on paper, filtration through paper, filtration through paper filters must be avoided). If the more or less clear solution so obtained contains no physiologically unacceptable substances, it can be used as such for the products of the process. Other dissolved substances that the extract contains, including the solubilizing agent, are removed wholly or partially by methods known as such for the separation of protein materials, which gives a further concentration. Such methods are precipitation with acetone, ethanol, or ammonium sulfate, and techniques such as gel filtration and ion exchange. The purity of the concentrates can be checked electrophoretically.

These concentrates can be used in the products when any physiologically unacceptable solubilizing agents have been completely removed. If desired, the concentrates can be concentrated further or evaporated to dryness at a temperature of not greater than 30°C., preferably room temperature or lower: for example, concentration or evaporation to dryness is carried out in a thin-film evaporator or by freeze drying the frozen solution in high vacuum.

The concentrates obtained by dissolving the active factor with the aid of solubilizing agents of the first group contain the active factor in a form having an isoelectric point between about 4 and 7; the form of the active factor obtained with the aid of solubilizing agents of the second group has a lower molecular weight and an isoelectric point of about 9.

From a solution of the active factor obtained by means of solubilizing agents of the second group, a preparation has been obtained which exhibits only one band in the analytical ultracentrifuge. This preparation, which has no taste of its own but still shows the sweetening activity, is therefore the almost pure active factor, which will be called miraculin. The processes give a yield of up to 100 mg. of miraculin from 1 kg. of berries.

The molecular weight determined by means of the analytical ultracentrifuge is about 42,000 (±3,000). On hydrolysis, amino acids and saccharides are formed. Saccharides have been detected in the band of miraculin after electrophoresis on polyacrylamide gel by oxidation with periodic acid and by the Schiff method. This also confirms the finding of Inglett et al. that miraculin is a glycoprotein.

The isoelectric point is about 9. Miraculin is tasteless and heat-labile, but substantially stable at pH values between 3 and 12. However, when it is allowed to stand for two hours at room temperature at a pH of 2, the sweetening activity is largely lost. It is attacked by proteases, losing the sweetening activity. Miraculin proved to be homogeneous both in sedimentation analysis in the ultracentrifuge and on gel filtration through modified dextran gels.

The physiologically acceptable concentrates and miraculin itself can be added to various

foodstuffs and beverages such as yoghurt, buttermilk, junket, mayonnaise, fruit juices, jam or marmalade. The amount of concentrate or active factor to be incorporated depends on the miraculin content and on the foodstuff or other product. It can be determined rapidly by a simple test. The addition of almost pure miraculin to yoghurt, for example, in an amount of 2.5 mg./l. gives a sweet taste. With smaller amounts, the sour taste is reduced, but the sweet taste is not yet present. In berry juice, about ten times as much is needed: 20 ml./l. gives the sweet taste.

The stability of the substance in milk products is surprisingly good: after four days at room temperature or 14 days at 4°C. the same activity was found. The stability in berry juice was less good, however, which is ascribed to the lower pH of the juice (pH 2.8). Preferably, the products should have a pH value higher than 3.

Example 1: 1.1 to 2.2 g. of fruit flesh (wet weight) from 2 to 4 g. of berries of *Synsepalum dulcificum* was homogenized with a 1% solution of polyethyleneglycol (mean molecular weight 20,000) in water. The pH of the homogenate was brought to 7 with a saturated sodium carbonate solution. Finally it was centrifuged at 10,000 *g* for 30 min. The liquid obtained in this way was treated at 20°C. with an equal volume of acetone with stirring. The precipitated active factor was centrifuged off for 10 min. at 1,200 *g*. The precipitate was washed with two volumes of acetone-water (2:1) and taken up in 6 ml. of 0.1 M potassium phosphate buffer, pH 7. Insoluble material was centrifuged off at 10,000 *g* (30 min.). The resulting solution, when added to 1 l. of yoghurt, imparted a pleasant sweet taste to this product. The same result was obtained by extraction with a 5% solution of polyethyleneglycol (mean molecular weight 400) in water, 1% of caffeine in 0.1 M potassium phosphate, pH 7, 0.1% of sorbitan mono-oleate dipolyethylene glycol ether in 0.1 M potassium phosphate, pH 7, and with 3% of peptone in water.

Example 2: 4.4 to 8.8 g. of fruit flesh (wet weight) from 8 to 16 g. of berries was homogenized with 20 ml. of a 1% gelation solution in water or in 0.1 M potassium phosphate buffer, pH 7. The pH of the homogenate was brought to 7 with a dilute KOH solution, the mixture was centrifuged for 30 min. at 10,000 *g*. The solution was purified further by gel filtration through Sephadex G-25, a modified dextran [swollen with water and filled into a 3 x 11 cm. column (volume of the bed 78 ml.)]. The active factor (25 ml.) was percolated through the column with water at 150 ml./hr. The eluate with volumes 25 to 50 ml. inclusive contained the active factor; it was used for sweetening purposes.

Example 3: 2.2 g. of the fruit flesh, wet weight, was homogenized with 5 ml. of a 1% solution of salmine sulfate in water. After homogenization, the pH of the mixture was 3 to 3.5. The resulting mixture was centrifuged at 10,000 *g* (30 min.)

Similar results were obtained when the pH of the mixture after homogenization but before centrifuging was brought to values of up to 10. The pH value required for the further treatment of the extract can therefore be adjusted before or after the insoluble constituents are centrifuged off. The extract obtained in this way contained a large amount of salmine, which was removed either by ion exchange or by gel filtration.

The removal of the salmine by ion exchange proceeded as follows. The pH of the solution was adjusted to about 10, and the solution was percolated through a column of CM-Sephadex C-50, a carboxymethyl derivative of modified dextran which possesses cation-exchanging properties.

The CM-Sephadex C-50 was washed with 0.02 M sodium glycinate buffer (pH 10.5) and filled into a column (1 x 34 cm., volume of the bed 27 ml.). The solution of the active factor was added to the column and was percolated with the same buffer at a rate of flow of 25 ml./hr. The active factor was present in the eluate with elution volumes of 16 to 35 ml., inclusive. The solution was free from salmine (according to starch gel electrophoresis at pH 4.3).

The removal of the salmine by gel filtration proceeded as follows. The solution of the