Report of the Chief of the Bureau of Agricultural and Industrial Chemistry Agricultural Research Administration

1949

UNITED STATES DEPARTMENT OF AGRICULTURE

Report of the Chief of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, 1949

LETTER OF TRANSMITTAL

United States Department of Agriculture,
Washington, D. C., October 12, 1949.

Dr. P. V. Cardon,
Agricultural Research Administrator.

Dear Dr. Cardon: I present herewith the report of the Bureau of Agricultural and Industrial Chemistry for the fiscal year ended June 30, 1949.

Sincerely,

G. E. Hilbert,
Chief.

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INTRODUCTION

The following pages report some of the more important accomplishments of the Bureau of Agricultural and Industrial Chemistry during 1948–49 relating to the utilization of agricultural commodities. Ninety-one items, having informative headings, are grouped under 19 comprehensive titles that indicate the commodities, commodity classes, components, products, processes, or research tools with which the items are concerned.

In addition to telling what was learned through fundamental chemical and physical research on particular agricultural commodities, components, or derivatives, this report summarizes the progress made in certain laboratory and pilot-plant experiments designed to develop new or improved products through chemical processing of farm commodities or wastes. Brief items are also included regarding commercial applications during the year of research results previously obtained in the Bureau's work.

Most of the items relate to projects that were supported by the 1949 Agricultural Appropriation Act funds specified for the regional research laboratories and for agricultural chemical and naval stores investigations of this Bureau. Some relate to projects supported by the Special Research Fund authorized by the Bankhead-Jones Act of June 29, 1935, the Strategic and Critical Materials Stock Piling Act of July 23, 1946, or the Research and Marketing Act of 1946. Unless otherwise indicated, however, the work reported was done under regular Bureau projects.

Research has begun under 12 new Research and Marketing Act projects since preparation of the last report. Results obtained thus far under 5 of these new projects are discussed in the present report, together with those obtained under 14 of the 35 other Research and Marketing Act projects started previously. One RMA project was completed with the construction of the research laboratory at Pasadena, Calif., which was dedicated on April 14.

Further and more detailed information regarding much of the Bureau's work may be found in the 325 publications that were issued during the year. Most of these were research papers, which appeared in scientific and technical journals. Information on newly developed processes and products is given in the specifications of the 98 Government-controlled patents granted during the year to employees of the Bureau. Both publications and patents exceeded in number those of 1948, which in turn exceeded those of any previous year. As compared with the fiscal year 1948, there were 5 percent more publications and 98 percent more patents. A mimeographed list of the publications issued and patents granted during the fiscal year 1949 is available.

In planning and conducting its research work, the Bureau's administrative staff and project leaders have been guided by numerous conferences with representatives of agricultural, industrial, commercial, and scientific organizations. Consideration has been given to the

factors of need, timeliness, prospect for achievement of the goal, the relation of proposed new projects to those already under way, the priority of proposed projects, and the relation of proposed projects to research under way in other research organizations. The Bureau has cooperated with other units of the Agricultural Research Administration, with other branches of the Department, and with other agencies of the Federal Government. It has cooperated also with the State agricultural experiment stations, with colleges and universities, with associations of producers, sellers, and processors of agricultural commodities, and with individual firms interested in making or using new products that can be obtained from agricultural sources.

In order to provide for regular consultation and discussion in matters pertaining to the nature, policy, and administration of its research, the Bureau arranged for memorandums of understanding between each of the Regional Research Laboratories and all of the State agricultural experiment stations within the region shortly after each laboratory was opened. As a result, conferences between representatives of the experiment stations and Bureau administrative officers have been held annually.

In addition to the general memorandums of understanding between the Bureau and the State agricultural experiment stations, there are special memorandums of understanding or cooperative agreements with some of the State experiment stations, as well as with cooperating Government agencies, nongovernment research organizations, educational institutions, and individual or organized producers or industrial consumers of agricultural commodities. At the close of the fiscal year 1949, 81 special memorandums of understanding and 14 cooperative agreements were in effect. Twenty-eight of the understandings and agreements were with industrial firms or associations, including seven food packers, and four were with industrial research organizations. Three firms cooperated with the Northern Regional Laboratory in research on the improvement of the quality of strawboard, one of which detailed a scientist to work on this problem in that Laboratory.

A manufacturer of cottonseed oil cooperated in the research of the Southern Regional Laboratory on cottonseed and cottonseed products; another cooperated in solvent extraction; and a third in an investigation on prevention of deterioration in moist cottonseed during storage. One scientist was sent to the Eastern Regional Laboratory by the Sugar Research Foundation to investigate the industrial possibilities of allyl sucrose; another was sent to the Northern Regional Laboratory by the Corn Industries Research Foundation to study the isolation of isomaltose from starch; another was sent to the Agricultural Chemical Research Division by the National Confectioners' Association to participate in research on nutritionally balanced candies, and two others were stationed at the Southern Regional Laboratory by the National Cottonseed Products Association to investigate the safe storage of moist cottonseed and the removal of plant pigments from cottonseed products.

The Bureau has also cooperated with foreign governments interested in the utilization of agricultural commodities through chemical processing. During the past year 15 trainees from foreign countries were allowed to work in the Bureau's laboratories on problems of mutual interest, and some of them were joint authors of papers offered for publication in scientific or trade journals.

On September 30, 1948, Louis B. Howard resigned as Chief of the Bureau to become head of a newly organized department of food technology at the University of Illinois. He was succeeded by the present Chief, and the position as Director of the Northern Regional Laboratory, vacated by him, was filled by the appointment of R. T. Milner, formerly head of the analytical and Physical Chemical Division of the Northern Laboratory.

In order to relieve the Chief of the Bureau from numerous time-consuming details of administration and to give more opportunity to fulfill the primary functions of his office, a system was instituted under which only the three assistant chiefs of the Bureau and the four directors of the regional research laboratories report directly to the Chief of Bureau. Each director is a special representative of the Chief of the Bureau as regards instructions to and reports from members of the Bureau's staff in the entire region, including not only those in his own laboratory, but also those in other Bureau divisions and project units within the region. In the Washington area (including Beltsville, Md.) each division head or Assistant to the Chief reports to one or another of the three assistant chiefs as instructed.

A Bureau Research Council, comprising the chief (as chairman), the three assistant chiefs, and the four regional laboratory directors, was organized to meet at the call of the chief to advise in developing and promoting the research program of the Bureau, and to participate in making policy decisions. These meetings have supplanted the former semiannual directors' meetings held in Washington.

Personnel administration was also decentralized, except for the Washington area, the business manager of each regional laboratory being designated as a regional personnel officer with authority to appoint, grade, and rate the efficiency of employees within the entire region.

COTTON AND COTTON PRODUCTS

Water-Resistant Fabrics Produced From Specially Selected Cottons

As an approach to the production of watertight cotton fabrics, the Southern Regional Research Laboratory has utilized the long known capacity of cellulose, which constitutes more than 90 percent of cotton fiber, to swell when wet. As the wet fibers swell, they close up the pores of closely woven fabric and thus enable it to stop the passage of water. A few years ago the Southern Laboratory succeeded in utilizing this self-sealing-by-swelling effect in the production of a watertight all-cotton firehose. In that product the swelling was aided by coating the yarn with a chemical derivative—hydroxyethyl ether—of cellulose.

Later, successful application of the self-sealing principle to shirting and other lightweight cotton fabrics inspired extensive research on the swelling properties of cotton fibers. It was not known to what extent cotton fibers swell or whether the degree of swelling differs in different kinds of cotton. In recent work under an RMA project attention was centered on the parts played by the structures of the fiber, the yarn, and the fabric in the final water stoppage. This knowledge is necessary for the best utilization of the self-sealing effect in the commercial production of watertight cotton fabrics.

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Work on the problem disclosed that the ability of wet cotton fibers to seal the pores of a fabric is closely associated with the degree of immaturity of the cotton, as indicated by the proportion of thin-walled fibers. It had been determined conclusively that immature (thin-walled) fibers have the greatest closing ability, even though variations in the cross-sectional swelling of fibers with moisture changes are small for all kinds of cotton. The three principal techniques used to acquire this information were the orifice test and the microscopical measurement, both mentioned previously, and a new technique based on the rate at which fibers dry after being saturated with water.

Tests on fabrics woven experimentally at the Southern Laboratory from cottons selected to illustrate the effects of differences in thickness of the fiber walls showed appreciable advantage from the use of cottons of lower maturity and a pronounced improvement by weaving yarns of immature cotton into extratight fabrics.

The weaving of very tight fabric—25 to 30 percent more dense than other weaves—was accomplished by using a special loom attachment that was developed at the Southern Laboratory under its regular research project on mechanical processing of cotton. This development is important to the Army Quartermaster Corps for the manufacture of tents and tarpaulins and to the cotton textile industry for the production of certain industrial and special fabrics. Drawings of the special loom attachment were furnished on request to a number of firms that were interested in duplicating it.

Special Cotton Fabrics Have Ion-Exchange Properties

Ion-exchange substances are important in some industrial processes as filtration and reaction media for the removal of certain ions of dissolved salts from water or from solutions of desired products. Typical examples are the softening of water by the replacement of calcium and magnesium ions with sodium or potassium ions and the purification of sugar syrups by removal of certain mineral ions. The commercial ion-exchangers in current use are granular resins which by double decomposition exchange acidic substances (anions) or basic substances (cations) in alternating treatments of the solutions to be purified.

In recent research at the Southern Regional Research Laboratory several types of chemically modified cotton fabrics were developed which possess ion-exchange properties. The fabrics enter into the same reactions as the ion-exchange resins; they can be regenerated repeatedly for reuse; and they have an advantage in that their form permits them to be readily manipulated. They are particularly useful whenever it would be advantageous to have ion-exchangers in fabric rather than in granular form.

These special cotton fabrics are produced by introducing certain reactive chemical groups into the cellulose molecule to form new compounds having the desired properties. Aminized cotton, whose production by the action of 2-aminoethylsulfuric acid and sodium hydroxide was described in last year's report, has anion-exchange properties. Phosphorylated cotton, made by the action of phosphoric acid and urea, is a cation-exchanger. Partially carboxymethylated cotton, prepared by the introduction of carboxymethyl groups into

the cellulose, is another cation-exchanger. A fourth type of fabric being investigated is partially etherified cotton, produced by treatment with 2-chlorethylsulfonic acid and sodium hydroxide, which has cation-exchange properties similar to those of phosphorylated cotton.

Aminized and phosphorylated fabrics have been produced in considerable quantities in the pilot plant of the Southern Laboratory with standard textile processing equipment. They have been used as ion-exchangers in producing peanut protein of exceptional purity. The solutions used to disperse the proteins are made alkaline by treatment with the aminized fabric (anion-exchanger) and the protein is precipitated with the aid of phosphorylated fabric (cation-exchanger). With the fabric form of ion-exchanger, purer peanut proteins are obtained because nonprotein substances in the dispersion, such as phosphorus and other ash constituents, remain on the fabric when it is removed and thus are not precipitated with the protein.

The successful use of ion-exchange fabrics, which may be regarded as chemicals, introduces a new concept in the ion-exchange field. The special fabrics which already are in use as laboratory reagents should find several industrial applications.

Mineral Pigments Give Added Life to Tobacco Shade Cloth

Last year's report stated that sufficient tobacco shade cloth to cover several acres had been treated with lead chromate by the Southern Regional Research Laboratory and a private firm with which it cooperated and had been sent to Florida for service tests in tobacco fields. The effectiveness of this treatment for reducing damage by sunlight has been established in tests started in 1948 and continued in 1949 by cooperating growers of wrapper-leaf tobacco in northern Florida. At the end of the 1948 season the treated material had lost only about one-third as much strength as the untreated. Early reports during the second season's use were favorable enough to indicate that the cloth might prove serviceable for a third season. Preservation during three seasons would be significant, since deterioration of shade cloth by sunlight is rapid, and the necessity of replacement after only one season, as is the case with untreated material, involves heavy expense.

A hindrance to industrial application of the lead chromate treatment has been the difficulty of treating the 200-inch-wide cloth preferred by tobacco growers. Because it was difficult to process the wide, open-mesh fabric on standard finishing machinery the 10,000 yards used in the tests were treated in laundry machines. Recently one large finishing company has successfully used standard textile machinery to process 100-inch-wide material. It is planned to sew together two of these 100-inch strips to give the tobacco growers the width they desire.

Insect-Resistant Treatment Devised for Cotton-Bag Fabrics

Damage to flour during shipment and storage in cotton bags by insects that penetrate the fabric represents an immediate loss to millers and an ultimate loss to farmers. Cotton is losing ground to paper in the container field because of paper's greater resistance to penetration by insects. The problem therefore is to find a treatment that can be applied by textile plants in practical operations, will be effective

over several months of use, and will not contaminate the flour nor be objectionable from the public-health standpoint.

This complex problem was attacked jointly under an RMA project by the Bureau of Entomology and Plant Quarantine and this Bureau's Southern Regional Laboratory. It was found that certain treatments when applied to cotton cloth prevented insect penetration for 8 months. Pyrethrum alone or a mixture of pyrethrum with piperonyl butoxide is effective and can be applied to warp yarns with the size prior to weaving, or with the starch and filler during finishing. The added cost of the treatment is estimated at not more than 1 cent per square yard of cloth. Both of the substances mentioned can be used safely for this purpose.

There is a strong industrial interest in this development. Details have been furnished to the Textile Bag Manufacturers' Association, whose members are now conducting large-scale tests to determine the practical value of the treatment for commercial use.

Partially Acetylated Cotton Lasts Long in Hard Usage

More good reports have come from the fishing industry on the excellent rot resistance of partially acetylated cotton fishing gear during long immersion in both fresh and salt water. Acetylated twines, cords, and gill nets after 4 months' exposure in North Carolina river and ocean waters either retained all of their strength or lost only 15 percent of it, in contrast to a 50-percent loss of strength by cotton materials coated with tar, which in the past was considered to give the best protection. Untreated cotton subjected to these tests lost all of its strength within a month.

Besides having superior rot resistance, partially acetylated cotton has shown better heat resistance than ordinary cotton. Fabrics were acetylated at the Southern Regional Laboratory for trial in a laundry as pad covers on the presses, a use which obviously provides a good test of heat resistance. The first results showed that the acetylated covers lasted twice as long as ordinary pad covers. Later results indicated that much longer serviceability could be expected.

The laboratory's successful small-scale production of partially acetylated cotton textiles for many types of service tests has shown that the treatment can be given in batch operations on commercial machinery with only slight adjustments. More recent work on the treatment has been directed toward the development of a more practical, rapid, continuous process.

The great interest persistently shown by several industries in partially acetylated cotton clearly indicates the need for the commercial availability of such a product. The interest manifested recently by one of the largest cotton processors in the United States has encouraged the hope that commercial production of partially acetylated cotton products may be realized in the near future.

Dealers and Mills Use Dye Test for Immature Cotton

The report for 1947 described a dyeing technique developed at the Southern Regional Research Laboratory to distinguish between mature and immature cotton by a differential color effect. This test has attracted world-wide interest and has contributed significantly to

the scientific knowledge of cotton. It has been welcomed by the cotton trade as a possible answer to a need, long felt by fiber-testing laboratories and textile mills, for a simple and rapid method of determining the character of cotton with special reference to its maturity. When too many immature fibers are present in cotton, they cause difficulties in processing, raise the cost of manufacture, and lower the quality of most products. It is important to know in advance the proportion of immature fibers present, but the usual microscopic methods of judging maturity are too tedious and time-consuming for routine use in a quality-control program.

Several brokers are using the dyeing test in their laboratories to forestall trouble at the mill by avoiding shipments of immature cotton except for certain special uses. Some cotton mills are using the test for part or all of their cotton supply. In one mill the test is used to check the uniformity of the blend of immature fibers—nearly always present—with mature fibers. Another mill use is for detecting cotton bales that contain excessive proportions of the immature fibers which eventually cause "neppiness," or "spottiness," in yarns and fabrics. The test has also been adopted by cotton breeders in variety-development work.

The new test depends upon a difference in the attraction of two types of cotton fibers for two commercial direct dyes, one red and the other green, which are applied in the same dye bath. Well-developed (thick-walled) fibers retain the red dye, while the underdeveloped (thin-walled) fibers retain the green. Cottons having different proportions of mature and immature fibers will dye to different blends of red and green. When the test is used in a mill, a sample of cotton from each bale is taken, labeled, and put in the dye bath. The color of the sample after being dyed shows whether the bale contains a high proportion of immature fibers.

Research on the dyeing test for maturity of cotton was continued last year under an RMA project to adapt it as nearly as possible to the rapid quantitative estimation of immature fibers in cotton and to the solution of other problems that arise in the processing of immature cottons. A supplementary technique was developed to make the estimate strictly quantitative. It comprises mounting some of the dyed cotton on a glass slide and counting under a low-power microscope the number of red and green fibers within a marked area. This technique has the advantage over the usual microscropic methods of eliminating the problem of "borderline" fibers, or fibers of questionable maturity.

Lint Cutter Becomes Basic Unit in New Process for Using Waste Cotton

A machine for cutting lint cotton to short-length fibers suitable for the manufacture of nitrocellulose in equipment designed for linters was developed by the Southern Regional Research Laboratory as an emergency measure in World War II. The report for 1946 described the successful performance of a model of the machine at a plant manufacturing chemical cellulose. Some time after the war ended, the machine was declared surplus property by the War Production Board and was dismantled. In 1948 it was purchased from a used-equipment dealer by a manufacturer of specialty paper pulps. Re-

cently, the machine, modified according to recommendations of the Southern Laboratory, was installed in a plant where it is the basic unit in a new process that uses waste cotton for the manufacture of partially digested cellulose pulp. The plant will probably be in full production before the end of 1949 and is designed for an output of 48 tons of pulp daily, or more than 15,000 tons annually. This industrial application of the lint cutter has broad implications. It may open up a new field of utilization not only for cotton waste but also for short-staple, low-grade cotton lint, if a surplus should accumulate.

This development furnishes an example of the application of the Bureau's wartime research in meeting industrial peacetime needs.

OILSEEDS AND THEIR PRODUCTS

Knowledge of Changes in Cottonseed Aids Proper Storage

Information of considerable value was obtained by the Southern Regional Research Laboratory in its investigation of the chemical and physical changes that occur in cottonseed as the harvesting season progresses. The data will be very helpful in determining the most satisfactory conditions of storage for seed harvested under various conditions.

Analyses revealed a definite relationship in the trends of several important characteristics of cottonseed taken from a gin at intervals during 1948. The contents of free fatty acids, ammonia, and free amino nitrogen all rose as the season progressed, indicating increased break-down of both oil and protein. Seeds in which both these components have begun to break down heat more rapidly and to higher temperatures than do sound seeds, thus deteriorating to a greater extent. If the degree of break-down in the seed is known at the time of storing the best conditions for economical handling can be selected for any particular lot of seed.

Chemical treatments similar to those that proved effective in storage of mill-scale lots of cottonseed prior to processing successfully inhibited the heating and further deterioration of all samples used in this study. Comparative germination tests on treated and untreated seeds that had been stored under identical conditions showed that the treated seeds retained viability to a greater degree.

Moist Flaxseed and Rice Stored Safely After Chemical Treatment

Increased production of winter-grown flaxseed in certain areas of Texas during recent years has given the South a foothold on a new oilseed industry. However, the future of this industry appears to depend on successful methods of storing the seed in a climate conducive to rapid deterioration. Oil-mill operators in the flaxseed-producing area, who were familiar with the successful application of chemical treatments to prevent the spoilage of cottonseed in storage, asked the Southern Laboratory to assist them in solving the flaxseed-storage problem.

Treatment with a mixture of propylene glycol dipropionate and bischloromethylxylenes, without additional handling, was as successful in controlling heating and deterioration in preliminary tests on mill-scale lots of flaxseed as was mechanical cooling, the method in

general use. When the flaxseed was treated with as little as 10 pounds of the chemicals to a ton of seed and this treatment was supplemented by a moderate amount of mechanical cooling, the results were much better than with chemicals or mechanical cooling alone. Seed containing 10 percent of moisture kept for several months under these conditions. Shorter tests with seed of higher moisture content, up to 22 percent, also were successful.

Another southern crop of increasing economic importance is rice. But it has faced serious storage problems in recent years because of the increasing use of combines for harvesting and the resulting high moisture content of the grain. Several chemical compounds were found to inhibit heating and to reduce carbon dioxide production and the formation of free fatty acids, but some of these caused an off-odor and a metallic taste when the rice was cooked. Finally two chemicals were found which not only inhibited deterioration during storage but also imparted no objectionable odor or taste during and after cooking. They are acetic acid and sodium metabisulfite. As was the case with flaxseed and cottonseed, chemical treatment supplemented by short periods of mechanical cooling gave better results than did the use of mechanical cooling or chemicals alone.

Stability of Rice Bran and Rice-Bran Oil Improved

During the past year the Southern Regional Research Laboratory made considerable progress under an RMA project in solving some of the problems involved in the utilization of rice bran (the mixture of rice germ and natural coating removed from brown rice during the milling process) as a source of high-quality edible oil. The most serious disadvantage of rice bran for this purpose is the formation of fatty acids in it is so rapid that after a short time the oil is not worth extracting.

In its search for ways to improve the quality of the extracted oil, the Laboratory determined the various factors that contribute to the formation of fatty acids in the bran during storage and applied this knowledge in developing better storage methods. The investigation revealed that the rate at which free fatty acids form can be retarded by lowering the temperature at which the bran is stored, and that brans from some varieties of rice require a lower temperature than others for safekeeping. Drying the bran before storage and maintaining a low moisture content during storage greatly prolong its keeping period. By observing these precautions it was possible to store rice bran safely for 7 months, whereas untreated rice bran would keep only a few days.

Oil extracted from high-quality rice bran and processed by the conventional methods of refining, bleaching, hydrogenation, and deodorization was equal in quality to edible oils from other plant sources. The most outstanding characteristic of refined rice-bran oil is its exceptional resistance to the development of rancidity. Cooking oils and shortenings processed from rice-bran oil kept twice as long as did similar products made from other vegetable oils. Furthermore, additions of rice-bran oil to other oils improved the keeping quality of the resultant products. The ease with which rice-bran oil can be winterized makes it particularly suitable for use in mayonnaise and as a salad dressing.

New Gossypol Assay Aids Cottonseed Industry

The returns from cottonseed cake and meal would be substantially increased if processing methods that improve their nutritional quality could be developed. At present when either hydraulic or screw pressing is used to recover the oil, the cottonseed meats, with or without added moisture, are cooked prior to pressing. When solvent-extraction methods are used, the resulting meals are generally heated or toasted to reduce to a low value the amount of free gossypol and related substances that exist in raw cottonseed. Knowledge of the amount of these susbtances remaining in cake and meal processed by different methods, and under varying conditions of the same method, could guide the choice of optimum conditions for cottonseed processing to yield the most nutritious meal. For this reason, a rapid and accurate method for the determination of free gossypol in cottonseed cake and meal was greatly needed.

Such a method was developed at the Southern Regional Research Laboratory during the past year. By its use the total amount of free gossypol and gossypollike substances in either cottonseed or cottonseed products can be rapidly and precisely determined. Though similar in principle to some older methods, it is much simpler. The gossypol pigments are extracted with acetone, and a characteristic color for photometric evaluation is developed by reacting them with para-anisidine.

This method has been adopted tentatively by the American Oil Chemists' Society. It is used by a number of industrial laboratories for the control of cottonseed processing and in research on the development of improved processing methods. It has been welcomed as a useful tool for such purposes.

Pure Gossypol and Gossypol-Protein Produced for Investigation

Ever since the Southern Regional Research Laboratory's unique solvent-fractionation process made possible the separation of pigment glands from cottonseed, the need for information on the physiological properties of the pigments in these glands has been growing. Although preliminary tests indicated that some of these pigments might be useful in the pharmaceutical field, data vital to the development of such uses were not available, partly because sufficient supplies of pigment glands, and especially of the pure pigments, had not been made available for extensive investigations. During the past year, therefore, an effort was made under an RMA project to increase the supply of these pigments, particularly gossypol, by laboratory processes.

A procedure previously developed for the isolation of pure gossypol from pigment glands was modified to permit 1-pound lots of pigment glands to be processed at one time. This development made it possible to produce a substantial amount of crystalline gossypol. Part of this quantity was used by cooperating laboratories in studying the toxicity of gossypol as compared with that of the whole pigment glands. These laboratories found that the pure gossypol had physical properties entirely different from those of gossypol held within the pigment glands, and the workers believed this variation in the physical state of gossypol might be a key factor in determining its toxicity.

To test this theory a way to prepare purified gossypol in a form that would resemble its physical state within the pigment glands was re-

quired. One of the main differences was that the pure crystalline gossypol would not disperse in water, whereas whole pigment glands mixed with water produced very fine suspensions of the pigments they contained. Efforts were made, therefore, to produce a water-dispersible form of gossypol. This was accomplished by combining gossypol with peanut protein. The resulting substance promises to be of considerable value not only in determining the effect of the physical state of gossypol on its pharmacological properties but also as a practical means of utilizing gossypol. Fairly large quantities of gossypol-protein are being prepared for future study by schools of pharmacology and by industrial organizations.

Cottonseed Protein Modified for Fiber and Other Nonfood Uses

Although cottonseed meals are recognized as a potential source of protein for industrial uses, the protein obtained from them in the past has been too dark for such uses. Even when the lightest-colored cottonseed meals were treated in the usual manner for extracting protein—with an alkali to dissolve protein and after filtration with an acid to precipitate it—the product was almost black. Thus the industrial utilization of cottonseed protein was severely handicapped, while consumption of proteins from competitive sources, particularly soybeans, rose steadily during recent years.

Last year the Southern Regional Research Laboratory developed a process that promises to remedy this situation. By washing cottonseed meal with weak hydrochloric acid, extracting the protein with a sodium chloride solution, and precipitating it with dilute sulfuric acid, a protein pale enough for industrial uses was obtained. In addition, ways were devised for stabilizing the protein by the addition of certain reducing sugars. These make possible the preparation of concentrated protein solutions with "working life" long enough to permit their use in adhesives, sizes, coatings, and fibers.

Even when its color and keeping quality are satisfactory, however, cottonseed protein has properties that make it unsuitable for spinning in its natural state. Methods found to be highly satisfactory for preparing fibers from proteins of soybeans, peanuts, and other vegetable sources completely failed when applied to cottonseed protein because of its tendency to jell or lump. Not until a method was developed for overcoming this tendency was the production of a synthetic fiber from cottonseed protein possible.

After considerable study the Southern Laboratory discovered that treatment with acid would change the structure of the protein so that it could be spun by the methods generally used in spinning other protein fibers. In 1948 enough fiber was prepared from acid-treated cottonseed protein to determine some of its properties. It has a yellow to light-orange color, feels soft to the hands, and has good dyeing characteristics. When dry it is about three-fourths as strong as wool and when wet it retains about 40 percent of its dry strength.

Advances Made in Solvent Extraction of Oilseeds

Producers of vegetable oil by solvent extraction have made practical use of data obtained by the Southern Regional Research Laboratory in two investigations relating to the recovery of solvent from the oil-

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solvent mixture by heating. These data have been supplied on request to nearly 100 firms and organizations in the United States and are being used regularly by operators of commercial solvent-extraction plants handling cottonseed, peanuts, and soybeans, and by manufacturers of evaporators and strippers for such plants. The information has also proved valuable in research on the design and operation of pilot-plant equipment for solvent extraction of cottonseed and peanuts. The two investigations are described below.

Better control of solvent removal

Boiling-point determinations were made on various mixtures of cottonseed oil and peanut oil with a petroleum solvent (commercial hexane). The recorded data have made possible more exact design of evaporative equipment and the proper selection of temperature and pressure in factory operations for removing solvent from the oil.

When the oil-solvent mixture (miscella) is heated to evaporate and recover the solvent, its boiling point rises as the proportion of solvent decreases. Theoretical calculations of the boiling point-vapor pressure-composition relation will serve for miscellas containing up to 50 percent oil by weight. But with an oil content above 50 percent the deviations from theoretical are appreciable. With an oil content approaching 100 percent—the region where temperatures are high and control critical—theoretical calculations are altogether unsatisfactory. The only data of value to guide the control of pressure, temperature, and time are those obtained by careful experimentation.

Reducing color fixation by extracted oil

The difficulty of producing refined and bleached cottonseed oil of prime color arises from the escape of coloring matter from pigment glands embedded in the meats and fixation of such coloring matter by the oil. Cooking of the meats virtually solves the problem for hydraulic-pressed cottonseed oil, and to a lesser extent for the screw-pressed oil, because heating makes the pigments combine with the protein. But in the solvent-extraction process the meats are not cooked; so more color gets into the oil.

The Southern Laboratory found that fixation of color by extracted cottonseed oil can be reduced by proper control of heat during the removal of solvent from the oil-solvent mixtures. Practical experiments demonstrated that color fixation can be held to a minimum when certain limits of temperature and holding time are observed in the handling of the miscella. Cottonseed oils prepared with these precautions were refined to yield oils of prime color. The finished oils were equal in quality to high-grade hydraulic-pressed oils.

New Process Yields Light-Colored Peanut Protein

A new process for treating peanuts, which removes 99 percent of the skins, was developed on a pilot-plant scale at the Southern Regional Research Laboratory. It comprises water-dipping, drying, and scalding of the peanut kernels before their solvent extraction. The dark-colored skins have always complicated the processing and utilization of peanuts, because they color the products. Elimination of this undesirable pigmentation by removal of the skins makes pos-

sible the production of light-colored protein of enhanced nutritional quality and greater potentiality for new industrial uses. The peanut protein that is free from pigments yields food products of good flavor and proper taste. A light color is also essential in the protein used for the manufacture of fibers since the textile products are often required in pastel shades.

The wetting-drying-scalding process is superior to two other processes that were previously developed to produce light-colored peanut protein. One separated up to 90 percent of the skins by blowing air through a stream of kernels that had been heated enough to rupture the skins, and the other overcame the pigmentation difficulty by dissolving nearly all the color from the skins by dipping the kernels in a solution of lye. The new process provides more complete removal of color than the first and causes less injury to the protein than the second. Thus far U. S. Grade No. 1 Spanish peanuts have been used in developing the process. Work is under way to adapt the process to lower grades of peanuts.

Peanut-Butter Processing Investigated

While peanut butter has been produced commercially for more than 50 years, very little information is generally available about the technology of processes or the effects of individual processing variables on the quality of the finished product. Because of the industry's need for such knowledge, an investigation was started at the Southern Regional Research Laboratory during the past year under an RMA project to provide information that will permit production of peanut butters of higher consumer acceptance.

So that the investigation might proceed under conditions as nearly as possible like those existing in commercial peanut-butter plants, batch-type grinding equipment of 250-pound capacity was installed at the laboratory. Special features of this equipment are a multiple proportional feeder attached to the peanut grinder to permit addition of hard fats and other solid materials to the peanuts, and a special pump and variable-speed drive to permit addition of a fluid stabilizer. Experimental batches of peanut butter are being produced from roasted peanuts alone and from the nuts with addition of various stabilizers and other ingredients to determine their effects on quality.

A thorough study of each controllable variable in the operation of the plant as it influences the quality of the finished product is in progress, and useful information on the effects of roasting at various temperatures has already been obtained. The soluble-nitrogen content of peanuts given a light roasting is definitely higher than that of darker-roasted peanuts, indicating progressive alteration of the protein as roasting conditions become more severe. In addition, the content of thiamin (vitamin B_1) becomes progressively lower as the nuts become darker from continued roasting.

Food Uses of Soybeans Studied in the Orient

Because of the continued demand for high-protein foods in this country the Northern Regional Research Laboratory sent a scientist to China, Japan, and Korea during the summer of 1948 to investigate Oriental methods of using and processing soybeans for food. Food

habits in the Orient are widely different from those in America. The high density of population has forced the people, especially peasants and laborers, to a vegetarian diet. Consequently there is need for high-protein food products, which soybeans supply. The Orient's special methods for using soybeans do not resemble practices in this country with soybeans, navy beans, or other bean types. Oriental people modify the soybean for table use by processing methods that separate the protein from the carbohydrates or by fermentation processes that impart desirable flavor.

The foods studied included: Soybean sprouts; soybean milk, curd (*tefu*), and cheese; soybean paste (called *miso* in Japan); soy sauce; monosodium glutamate (*ajinomoto* in Japanese); and fermented soybeans. Each of these products is important in the Oriental diet for supplying either protein or a special meat flavor that adds palatability to the food.

The two soybean products used most extensively in China are soy sauce and soybean curd. The sauce, still produced on a small scale by old-fashioned methods, imparts a meat flavor to vegetable dishes and supplies nutrient in the form of amino acids. The generally flavorless curd is a concentrate of protein and fat. It may be cut into small squares for use in soups, or it may be fried, smoked, boiled with vegetables, baked, or made a part of numerous other dishes.

In Japan, the use of soybean paste (*miso*) exceeds that of soy sauce, but considerable soy sauce is produced. The Japanese are more advanced in their food-processing methods than the Chinese, and one soy-sauce plant has reported a yearly production of 23,000,000 gallons. The Japanese are now developing chemical methods to replace the slow fermentation process for producing the sauce. The economy of food utilization in the Orient may be studied with profit by countries having food-shortage problems.

The results of this survey should contribute materially to the work at the Northern Laboratory under a Research and Marketing Act project to develop new foods from, and new industrial applications for, soybean proteins. The micro-organisms used in the various fermentations were collected and returned to the Laboratory for further investigation. Incidentally, soybean-seed samples were obtained for the Bureau of Plant Industry, Soils, and Agricultural Engineering for use in plant-breeding research.

"Gelsoy"—New Food and Industrial Product—Made From Soybeans

"Gelsoy," a new protein product made from soybean meal, has been discovered and developed at the Northern Regional Research Laboratory. This discovery marks the first known instance of a gel being derived from vegetable protein. The new edible material is obtained by carefully spray drying a water solution of alcohol-washed, hexane-extracted soybean flakes. It has a bland taste, dissolves in water, gelatinizes when heated, and possesses excellent binding, firming, and whipping properties. Laboratory and practical-use tests indicate that Gelsoy may find wide use in special food products such as meringues, ice creams, and gels. Laboratory tests indicate that Gelsoy is also suitable for nonfood uses as an adhesive for sealing cork or paper to metal, for heat-sealing of paper and other packaging units, and for

preparing gummed paper and may possibly be used as a foaming agent in fire-extinguishing preparations. Trial runs by an industrial company have demonstrated that this material is satisfactory for sealing the cork liners in bottle caps.

Under a cooperative agreement with a local soybean processing firm, a small-scale plant for the production of Gelsoy has been constructed. Pilot-plant studies on the production of this new protein material and further investigation of its properties and potential industrial uses are in progress.

Improved Procedure for Analyzing Tung Fruit Aids Industry

The improved procedure for sampling and analyzing tung fruit mentioned in previous reports has now been developed to the point where some of its benefits to tung farmers and the tung-oil industry can be cited. Chief among these benefits is the sound basis for trading that is provided by an accurate determination of the oil content of tung fruit.

The procedure was developed in the Bureau's Tung Oil Laboratory in cooperation with industrial laboratories. After using it for the second year, commercial analysts reported that results are entirely dependable, and the Commodity Credit Corporation found it extremely useful in carrying out the tung program for 1947–48. After thorough testing, the American Oil Chemists' Society, whose methods are widely used in the buying and selling of oilseeds, has adopted the procedure tentatively for the analysis of tung fruit, which, of course, means wider acceptance and greater use by industry.

An improved sampling technique explains the increased accuracy obtained by this new procedure. A fairly large sample of the whole fruit is ground to provide a representative portion for analysis. This procedure eliminates errors that occur when carpels from a few fruits or a few individual fruit kernels comprise the sample.

Use of the whole fruit rather than the kernel in obtaining the more representative sample also avoids the problem of proper hulling and shelling. Continued research during the past year provided some very valuable information on the effects of different methods of grinding the whole fruit to provide the sample and also about the influence of such factors as moisture and hull content on the determination of oil by rapid, practical methods of analysis.

POTATOES AND POTATO PRODUCTS

Surplus Potatoes Yield Flour for Europe

The unprecedented demand for more than 400,000,000 pounds of potato flour to be exported to Europe afforded an opportunity during the past year to convert many bushels of surplus potatoes to special food uses. But this quantity was more than 10 times this country's normal production of potato flour. Therefore means had to be found for quickly adapting existing equipment to serve this purpose. Last year's report told how the Eastern Regional Research Laboratory had developed a method for utilizing idle double-drum dryers in distilleries to help solve this problem. Recently this Laboratory modified potato processing so that other existing equipment can be used to produce potato flour by simple, cheap methods.

A second method employs the rotary steam-tube dryers found in idle industrial alcohol plants. The process consists of washing and sorting the potatoes, grinding them in hammer mills, sulfiting them to prevent discoloration, and then drying after mixing them with some previously dried material to prevent sticking to the dryer. The dried product is of satisfactory color and when ground to pass a 70-mesh screen yields flour of the type being exported to Europe. This process was demonstrated at the Laboratory on a large pilot-plant scale, a dryer of industrial size being used. It aroused commercial interest and, as a consequence, this method is now being used to produce large quantities of flour. Through publications, correspondence, and field-service trips the Laboratory materially assisted in initiating the process commercially.

Although thus far the second process has been carried out only in plants already constructed for other purposes, cost estimates show that a factory built where stored potatoes are available could produce 17 tons of flour a day at $39 per ton, including all costs except those of the potatoes and selling the product.

It was also shown on a pilot-plant scale that direct-heat rotary dryers, for example the type used in drying alfalfa, can be used to produce potato flour. This is significant, because by permitting year-round operation of such dryers the cost of production would be reduced for both the alfalfa meal and the potato flour. A third method, using such dryers, is analogous to the steam-tube-dryer process, just described, in that some of the previously dried product is mixed with the freshly ground, sulfited potatoes to prevent sticking to the dryer. To minimize the formation of dust clouds in the dryer and the possibility of dust explosion, the dried product is passed over a 100-mesh screen to remove the fine fraction before being recycled. The cost of such a process would be about the same as that of the steam-tube-dryer process.

A fourth method, which should find favor in certain existing plants, entails slicing the raw potatoes, coating them with a little dry potato meal to prevent sticking of the slices to each other and to the dryer, sulfiting them, and then drying them in direct-heat rotary dryers of the type commonly used in drying alfalfa. The dried product is screened to separate the coating meal for reuse. The slices are then ground to flour fineness.

The methods mentioned above, excepting the one that employs double-drum dryers, can also be adapted to the production of low-cost feed from potatoes by simply omitting the sorting and sulfiting steps. More than 3 tons of potato feed have been prepared by the steam-tube-dryer process in the pilot plant at the Eastern Laboratory. This is being used in cooperative feeding tests with ducks by the New York Agricultural Experiment Station at Cornell University and the Bureau of Animal Industry. The cost of feed prepared by this method is approximately $24 per ton exclusive of the cost of potatoes and sales cost on the product. A plant built for producing 17 tons per day would cost about $80,000.

The research on conversion of potatoes to a stable form by drying has been of value not only in meeting the emergency demand for food in Europe but also in showing how potatoes can be cheaply processed to permit their utilization for feed and industrial purposes.

Commercial Potato-Flour Samples Analyzed

In connection with the program of the Economic Cooperation Administration, the Federal Government contracted for about 460 million pounds of potato flour during the latter half of 1948. Since the capacity for producing potato flour in the United States was approximately 30 million pounds a year (only 10 to 15 million pounds above normal domestic consumption), production facilities had to be expanded considerably. About 348 million pounds of potato flour was delivered under this program up to June 1949, when exhaustion of 1948 potatoes in storage stopped operations. Additional information on the composition of potato flour made by various methods and from different types of potatoes was needed to establish proper specifications for purchasing the product—particularly by Government purchasing agencies.

One hundred samples of commercial and experimental flour made from potatoes grown in the major producing areas were analyzed at the Eastern Regional Research Laboratory under a Research and Marketing Act project. The samples had total assimilable carbohydrate values ranging from 70.9 to 84.4 percent on the dry basis, with an average of 78.1 percent. Moisture content varied from 5.8 to 11.4 percent, with an average of 8.1 percent.

Considerable variation was found in the total ash content of the flour. It ranged from 1.4 to 6.0 percent and averaged 4.4 percent. Flours lowest in ash were produced by pressing ground potatoes prior to drying. A disadvantage of pressing is that much of the inorganic salt content, protein, and other soluble substances is lost in the press juice.

Analysis of the potato-flour samples for protein (Kjeldahl nitrogen $\times$ 6.25) yielded values ranging from 3.8 to 13.3 percent and averaging 10.2 percent. The lowest protein values, as with the ash determinations, were obtained on samples produced from potatoes that had been pressed before drying. Crude fiber values ranged from 0.4 to 4.6 percent with an average of 1.8 percent. Crude fat (ether extract) values varied from 0.02 to 0.74 percent and averaged 0.27 percent.

The analytical data obtained on potato flours not only make possible the preparation of Federal specifications for the flour but are also useful in potato-utilization studies. The available literature on the composition of potatoes is old, and it is therefore helpful to have these up-to-date data, especially since newer potato varieties are now commonly grown.

Potatoes Converted to Important Industrial Acid

In the search for new nonfood uses for potatoes, studies were conducted at the Eastern Regional Laboratory under an RMA project to develop methods for preparing lactic acid from this agricultural raw material. Lactic acid is used extensively in the tanning, textile, pharmaceutical, and food industries and is potentially a valuable intermediate for the chemical synthesis of elastomers, plastics, and plasticizers. At present it is manufactured in the United States by fermenting sugars and sirups with addition of inorganic and organic nutrients. In Germany, potato starch has been used as the raw ma-

terial for lactic acid manufacure, but heretofore the acid has not been prepared directly from potatoes.

Washed potatoes were ground, cooked, and treated with enzymes similar in action to barley malt to convert the starch to sugars, which were fermented to lactic acid with no previous separation or purification. The acid was obtained in good yield after 110 to 120 hours of fermentation without the benefit of added nutrients, which are usually required when sugars or sirups are used. The enzymic conversion is not time-consuming, but progresses simultaneously with the acid fermentation.

Starch-splitting enzymes produced by the fungus *Aspergillus niger* NRRL 330 accomplished the conversion of potato starch to sugars. The mold was grown in some potato mash which was then used for converting a large batch. Likewise, the acid-producing *Lactobacilli* cultures were grown in a quantity of converted potatoes which was then used to inoculate a larger batch of converted potatoes. Of the three *Lactobacilli* tried, *L. pentosus* 124–2 gave the best yields of lactic acid.

The results showed that the potato has the carbohydrate, protein, mineral, and other constituents required for the growth of microorganisms, which provides a means for converting surplus and cull potatoes into lactic acid, a valuable industrial chemical. Together with earlier knowledge they suggest that the potato should be generally useful as a raw material for fermentation products.

GRAIN CROPS AND RESIDUES

Wet Milling of Grain Sorghums Not Hindered by Drying for Storage

Grain sorghums grown in the Southwest will soon be utilized at the rate of about 20,000 bushels per day for the production of starch and related products. This new and important industrial operation will be conducted on a year-round basis; therefore, large quantities of the grain must be stored. It is necessary that the stored grain contain not more than 13 percent of moisture if damage by molds and heat is to be avoided. Since most grain sorghum is harvested with the combine when its moisture content is comparatively high, it must be dried before it is stored. Experience with corn indicated that artificially dried grain sorghum might be difficult to process. Therefore investigations were conducted by the Northern Regional Research Laboratory in cooperation with one of the large processors of grain and the Texas Agricultural Experiment Station to determine the effect of artificial drying on the wet-milling characteristics of grain sorghums.

Samples of grain sorghum representing the varieties Hegari and Martin were harvested at three moisture levels, namely, high (20 to 25 percent), medium (17 to 18 percent), and low (15 to 16 percent), and were dried with air at temperatures of 125°, 150°, 175°, and 200° F. In some cases the final moisture content was 8 to 9 percent and in others, 12 to 13 percent. Samples of the dried materials were wet milled on a small scale by the conventional process, which includes steeping the grain in a water solution of sulfur dioxide, grinding and screening the softened grain to liberate the starch and recover the fibers, and tabling the resulting slurry to separate the gluten and starch fractions.

All samples of Hegari sorghum wet-milled well, and the yield and quality of products were satisfactory, thus indicating that no damage was sustained by the grain because of artificial drying.

Processing was satisfactory in most tests with Martin sorghum and good results were obtained. However, the samples which had been dried from low and medium moisture contents to 8 to 9 percent water appeared to be damaged. This was shown by the low starch yields obtained when the samples were milled and by the high protein content of the recovered starch. The adverse results do not correlate with temperature of drying, but appear to be related to the moisture content of the grain as harvested and to the extent of drying.

It was concluded that there is little danger of impairing the wet-milling characteristics of either early Hegari or Martin sorghum by artifiical drying with air at 125° to 200° F. if the grains are dried to no less than 12-percent moisture. The results of this research are of importance to the new wet-milling industry of the Southwest and to the farmers of that region who will produce the grain. Practical application of these results will encourage the industrial utilization of grain sorghums.

Mechano-Chemical Process Developed for Pulping Crop Residues

Experiments on the wet chopping of straw in preparation for cooking it with chemicals under increased pressure to produce paper pulp led to a simplified, economical pulping process that involves simultaneous mechanical and chemical action, eliminates the need for pressure-cooking equipment, and greatly reduces the time required for pulping.

Work at the Northern Regional Laboratory confirmed a previous observation at a strawboard mill that whole straw could be chopped effectively in the presence of cold water with the kind of commercial machine that is used industrially for disintegrating and hydrating old newspapers as a step in producing deinked paper pulp. In one experiment the wet-chopped straw was not immediately transferred to the pressure cooker containing the hot solution of pulping chemicals, but the hot solution was first added to the chopped straw in the disintegrating machine. The very rapid softening of the chopped straw suggested the possibility of completing the pulping in the disintegrator instead of in the pressure cooker. When an apparently satisfactory strawboard-type pulp was produced by churning the chopped straw in the disintegrator for only 1 hour in contact with the pulping solution, heated by steam injection, a series of experiments was carried out on producing both strawboard pulp and bleachable fine pulp in this way.

With the same quantity of lime and caustic soda and only 1 hour of cooking at atmospheric pressure it was possible to produce strawboard pulp that equalled in quality and yield the strawboard pulp produced by the current commercial practice of cooking straw for 5 hours under a pressure of 40 pounds per square inch.

Bleachable straw pulp for fine papers was produced by cooking the straw with 8-percent caustic soda and 4-percent sodium sulfide ("kraft" pulping chemicals) for only one-half hour at atmospheric pressure, against the usual requirement of cooking for 2 hours at a pressure of 100 pounds. Moreover, the yield of pulp was one-fourth greater, its color was much lighter, and the pulping solution could be reused three

times with addition of just enough chemicals to bring it up to its original concentration.

Neutral sulfite pulping chemicals (12-percent sodium sulfite and 3-percent sodium carbonate) did not work so well on straw in the disintegrating machine. Apparently they are too mild for pulping straw at the temperature produced by steam injection at atmospheric pressure.

The new mechano-chemical pulping process using the customary alkaline pulping solutions seems to be particularly well suited to pulping nonwoody lignocellulose materials like straws, flax and hemp tows, cotton, sugarcane bagasse, cornstalks, jute, reeds, manila, and sisal. The rapidity of pulping straw by this process at atmospheric pressure helps to overcome the disadvantage of straw's low density as compared with wood chips, which results in lower production per digester charge.

The new process is not believed to be feasible for pulping wood in the form of chips, but it should be possible to use it for pulping shredded wood, like fine excelsior, or coarse wood fibers, such as those produced for making insulating board.

Research Aids Marketing and Storing of Industrial Straw

The normal moisture content for keeping straw in good physical condition during marketing and storing is about 10 percent; moisture above this adds to transportation costs, and, if much above it, also brings about rotting. Until recently no accurate and practical field test for moisture content of straw was available. It had been customary for the straw buyer to examine and weigh a few bales and, on the basis of experience, make deductions in purchase price for the estimated amount of excess moisure. This method may lead to an estimate that is 5 percent too high and often has been considered by the farmer to be arbitrary and unfair. Much misunderstanding and dispute in straw marketing arose from the practice.

In 1947 the executives and straw buyers of the strawboard industry, the largest commercial consumers of straw, asked the Northern Regional Research Laboratory to devise a simple and practical test to replace this marketing practice. As a result, a small electrical instrument was developed for measuring moisture in straw and is now available commercially. It is simple to operate and has an accuracy of plus or minus 2 percent. This instrument is in general use by the various mills of the strawboard industry. Farmers have accepted the instrument's values with the feeling that they are now being paid the full value of their straw. Reducing the possible error of plus 5 percent in the moisture estimate by a straw buyer to plus or minus 2 percent in the moisture measurement by an instrument has meant an increase of several hundred thousand dollars in cash income to the farmers who supply straw. The mills now get all the straw fiber that they pay for and have, at the same time, a practical, rapid means for measuring the actual amount of straw used in their processing cycles. Another important result of this work is the greatly improved cooperation and mutual trust between farmers and straw buyers. Scientists at various experiment stations and others are investigating the use of this instrument for determining the moisture content of hays, baled cotton, sugarcane bagasse, flax tow, and similar fibrous materials.

Thus, both suppliers and consumers have benefited from this development.

Excess moisture in straw induces rotting, but even the purchase of dry straw does not solve the problem of storing straw in sound condition throughout the year. Straw is bulky and is used in such large tonnages by the mills of the strawboard industry that it must be stored in the open in bales formed into ricks of about 400 tons each. Until the late 1930's most of the industrial straw was baled from farm stacks in the fall, winter, and spring months, and only the dry straw of the stack was purchased. The amount of straw stored in ricks at the mill was relatively small, so loss to the mills in the form of rotten fiber was low. Now about 90 percent of the wheat is harvested with combines, and the whole year's supply of straw must be collected and piled for storage within about 60 days. The total amount of straw subject to rotting hazards while in the hands of the mill owners has greatly increased, and the present price of straw is about double that before World War II. In recent years the monetary loss from rotting of purchased straw was so high that some remedial measures appeared necessary. Under the leadership of the Northern Laboratory, a cooperative program was undertaken by seven of the strawboard mills and a number of chemical companies making fungicides.

A satisfactory solution of this storage problem was reached as a result of the first year's testing which was carried on under industrial conditions at each of the cooperating mills. It was found that about $70 worth of borax spread over the surface of the bales in the top two tiers of a 400-ton straw rick stopped rotting and that the bales at the end of a year's storage were sound, could be moved into the mill without breakage, and produced first-quality strawboard. A saving of $200 to $300 worth of straw per rick resulted from this work. This practice is now general. It is estimated that it saves about $200,000 worth of straw annually. Moreover the quality of strawboard has improved by elimination of rotten fiber as a raw material.

STARCH DERIVATIVES

Improved Allyl Starch Composition Developed

As reported previously, allyl starch has aroused great interest and attained some industrial use because its properties make it useful as a base for varnish or lacquer, printing ink, linoleum, and certain other plastic compositions. Although allyl starch has proved suitable for some uses, it has long been evident that some of its properties, particularly water resistance and toughness, must be improved further to expand the number and volume of its applications.

One possible method of improving the water resistance of allyl starch, namely, copolymerizing it with various reactive monomers, has been studied profitably at the Eastern Regional Research Laboratory. Because of its ready availability, favorable price, and hydrophobic character, styrene has received most attention thus far as a possible modifier for allyl starch. The copolymerization or "styrenation" is carried out at 65° C. in toluene-butanol solution with benzoyl peroxide as catalyst. Styrene constitutes 43 percent of the mixture. A suitably plasticized baked film of this product has excellent resistance to aqueous alcohol, dilute acetic acid, soapy water, distilled water,

and acetone, and good resistance to alkalies and boiling water. The plasticized film has an elongation of over 30 percent and a (Sward) hardness of 52. It compares favorably with the better commercial varnishes based on modified phenol-formaldehyde resin and air-drying modified alkyd resin, and is considerably better than unmodified allyl starch. Solutions of the styrenated product also have the advantage of greater tolerance for dilution with cheap hydrocarbon solvents than do similar allyl starch solutions of the same concentration.

The development of this improved allyl starch composition should make allyl starch more useful and easier to market. Moreover, it suggests that monomers other than styrene may be found useful in preparing improved allyl starch copolymers.

FERMENTATION AGENTS AND PRODUCTS

Culture Collection Aids World-Wide Research on Industrial Fermentations

Since good and sufficient tools speed the progress of almost any task, and micro-organisms constitute the tools of research on industrial fermentations, particular emphasis has been given to the development of a large and diverse collection of molds, yeasts, and bacteria at the Northern Regional Research Laboratory where the Bureau's research on fermentation is concentrated. Included in this collection are many cultures of proven importance to agriculture and industry; included, also, are other cultures of unknown potentialities which are being constantly evaluated.

The Bureau's culture collection now includes more than 5,000 strains, which are divided about as follows: Molds, 2,350; yeasts, 1,800; and bacteria, 950. In addition to these, which are maintained in active form, several thousand less intensively studied strains have been preserved in inactive form by drying without heating, but are available for use as needed. These dried (lyophilized) cultures constitute an invaluable reserve. During the past year one of the Bureau's scientists sent to the Orient to study food uses of soybeans brought back more than 500 soil samples as possible sources of new organisms for the culture collection.

The collection provides the cultures needed for various microbiological surveys, and the Laboratory's staff isolates or develops the selected strains essential for intensive investigation of particular fermentations. The work on penicillin during World War II was greatly aided by this collection. Initial investigations, which centered upon surface-culture studies, were carried out with Fleming's original strain of *Penicillium notatum*, but the first strain found to produce promising yields of penicillin in submerged culture (NRRL 832) was of entirely different origin and represented 1 of 36 strains of the *Penicillium chrysogenum* series contained in the collection that were available for penicillin-production tests.

Subsequently the laboratory staff made a wide and systematic search for additional representatives of this series and succeeded in isolating a superior strain, NRRL 1951. From this one, by natural variation and induced mutation, high-yielding substrains were produced which are still used for commercial production of penicillin throughout this country and abroad. Similar, but somewhat less dramatic, stories

could be cited regarding the microbes used in new fermentation processes for producing itaconic acid, riboflavin, and fungal amylase.

In addition to providing cultures for fermentation investigations at the Northern Laboratory, the collection also supplies authentic cultures to other Government agencies, to universities and hospitals, and to industrial laboratories engaged in microbiological research. Each year many cultures are sent to laboratories and culture collections in foreign countries. In 1948, for example, of the more than 1,600 cultures sent out from the Bureau's collection, 275 were furnished to Government agencies, about 500 to universities, colleges, and hospitals, 350 to industry, and almost 500 to laboratories outside the United States. It is thus obvious that the collection serves a purpose of far wider scope and significance than its immediate role as storehouse of cultures for the work of the Northern Regional Laboratory. It is, in fact, becoming a focal point for world-wide research on fermentations.

Concurrent with the search for, and the maintenance of, potentially valuable fermentative agents, studies on the biology and natural relationships of selected groups of micro-organisms are conducted. In 1945, Charles Thom and Kenneth B. Raper of the Northern Regional Research Laboratory published a comprehensive taxonomic and reference work on the industrially important mold genus *Aspergillus*. A similar study of the related genus *Penicillium* was subsequently undertaken, and a book by Raper and Thom was published in 1949. While fundamentally taxonomic in character, this book, like the earlier one on *Aspergillus*, provides an encyclopedic coverage of the biochemical activities of the agriculturally and industrially important Penicillia. A somewhat similar study of the yeasts is now in progress and this, too, will be published in book form when completed. Other studies of this type will be undertaken as the need for exhaustive investigation of micro-organisms belonging to particular groups develops.

Crystalline Alpha-Amylase Isolated From Malt

Most of the industrial uses of malt are based upon its ability to convert starches into dextrins and sugars. Thus in the manufacture of alcohol, starch is first converted to sugar by the use of malt, and the sugar is then fermented to alcohol with yeast. Although the most valuable component of malt is an enzyme (alpha-amylase) that converts starch to sugar, the Enzyme Research Division found that dry malt actually contains only about 1 part in 10,000 by weight of this active principle. For that reason the properties and behavior of the enzyme are very hard to determine with certainty by studying the malt itself.

During the year the Enzyme Research Division succeeded in isolating this active principle of malt in pure, crystalline form. This is the second amylase crystallized in this laboratory, the first one being beta-amylase which was isolated from sweetpotatoes. These preparations represent the only two crystalline amylases that have been made thus far from plant material.

The method of preparing the alpha-amylase was briefly as follows: A very concentrated malt extract was first heated and filtered to remove easily coagulated material. The enzyme was then precipitated from the filtrate by adding ammonium sulfate. The precipitate was filtered off and dissolved in dilute alcohol. Then powdered starch

was added to the alcoholic solution to absorb the enzyme. In this manner the enzyme could be removed from the solution in almost pure form. Further conditions were worked out later under which the enzyme was separated from the starch, concentrated by precipitation with ammonium sulfate, and finally crystallized. The enzyme is not stable except in the presence of small quantities of calcium ion, so calcium salts were added throughout the entire process of isolation. The crystalline enzyme apparently contains calcium as an integral part of its structure. Many of the properties of this enzyme protein were determined to get fundamental knowledge of value in later investigations.

It is now possible to observe under what conditions alpha-amylase is most active and under what conditions it is destroyed. Such information is of obvious importance to the users of malt. Many samples of the crystalline alpha-amylase were sent to industrial firms at their request for use in their own investigations. By using the pure enzyme it is possible to trace the formation of dextrins, which are intermediate compounds in the break-down of starch into sugar. The knowledge gained should lead to more accurate methods of manufacturing dextrins, some of which have properties that make them desirable as industrial sizing materials and adhesives.

Further investigation of the absorption of alpha-amylase by powdered starch showed that starches from various plants differ greatly in this respect. These observations seem to indicate differences in the susceptibility of the individual starches to enzymatic digestion.

Alcohol Tested as a Motor Fuel

Conversion of grains to alcohol for utilization as fuel for internal combustion engines appears to be the most promising large-scale industrial outlet for surpluses of such crops. Existing alcohol plants have sufficient capacity to produce annually about 300 million more gallons of alcohol than is produced for ordinary commercial needs. On the basis of 2½ gallons to a bushel, this is equivalent to 120 million bushels of corn. By comparison with a consumption of 30 billion gallons of motor fuel in 1948, 300 million gallons of alcohol is a very small quantity—only 1 percent. But if alcohol were used with gasoline as an antiknock component, the 300 million gallons would provide a significant power boost for more than a billion gallons of low-grade gasoline. Because of the possibility of future grain surpluses, as well as a prospective need for additional motor fuels, the Bureau has devoted a great deal of attention to the problems of producing alcohol from grains and agricultural residues and to the use of alcohol as a motor fuel. The work is centered in the Northern Regional Research Laboratory.

Two methods for using alcohol as a motor fuel are being studied. In one method dry alcohol is blended with gasoline before the fuel is put in the tank. In the other a mixture of alcohol and water is injected into the intake manifold during engine operation. Much of the fundamental data obtained for one method are applicable to the other. In both cases the favorable combustion quality of alcohol, in combination with high heat of vaporization, is the important factor.

Octane number, which is a measure of the combustion quality of a motor fuel, may be determined by the "Research" and the "Motor"

methods in the laboratory and by the modified "Uniontown" and "Border-line" knock methods on the road. The existence of four methods indicates the possibility of confusion in trying to predict adequately the service behavior of present-day motor fuels, which are made by so many different refining and blending processes. However it is possible to establish comparable octane ratings of alcohol blends, in spite of these difficulties of standardization, by using a number of hydrocarbon mixtures which resemble, on the average, the behavior of many commercial gasolines. For purposes of illustration, the Research octane numbers of a number of leaded and unleaded alcohol-gasoline blends have been found to be as follows: Base gasoline, 84.3; 10-percent alcohol blend, 90.1; 25-percent alcohol blend, 97.5; 35-percent alcohol blend, 100+; straight alcohol, 100+; base gasoline plus tetraethyl lead, 89.0; 10-percent alcohol, leaded, blend, 95.2; 25-percent alcohol, leaded, blend, 100+; and 35-percent alcohol, leaded, blend, 100+. In the last four cases, tetraethyl lead was added in the proportion of 0.5 milliliter per gallon.

A fuel having an octane number in the neighborhood of 100 is very much more valuable potentially than one having an octane number near 60. In general, however, an engine will not produce more power than is obtainable with a fuel that gives knock-free operation under maximum-power operation. Alcohol and other fuels having a high heat of vaporization are exceptions, since in some cases appreciable increases may be obtained through increase in volumetric efficiency. This applies especially to engines that operate at full power for long periods, as in truck operation, where as much as 7 to 10 percent increases in power were obtained, and in aviation engines, where power boost was frequently obtained without supercharge when alcohol-water mixture was injected, indicating that cooling of the intake air (increase in air density) resulted in higher power output.

The question of using tetraethyl lead in alcohol-containing motor fuels is of great importance, since its behavior in blends and straight alcohol differs considerably from that in gasoline. Small amounts of tetraethyl lead are very effective. Experiments have indicated that addition of 2 milliliters per gallon to blends containing 25 percent or less alcohol gives the greatest rise in octane numbers and that larger additions are less effective. This parallels the behavior of tetraethyl lead in gasoline. In blends containing more alcohol, however, little or no lead is tolerated. Lead has a negative action in straight alcohol, actually reducing the Motor-method octane number from 92 to 85. The presence of sulfur compounds in leaded gasoline lowers the octane number but does not have this effect when the gasoline is blended with 25 percent of alcohol.

There are no insuperable technological problems in the practical use of gasoline-alcohol blends as motor fuel. The most important points to be considered are stability of the blend, solvent action of the alcohol, and denaturant requirements. Stability is measured by the quantity of water that can be present in the alcohol without causing the alcohol and gasoline to separate into two layers. The higher the alcohol content, the more stable will be the blend. In Sweden a 25-percent alcohol blend has been used successfully. Before the blend can be used the fuel tank and feeding system of a car, particularly of an older one, must be thoroughly rinsed with the blend or straight alcohol to dissolve accumulated gum. The solvent action of the alcohol on the gum

will loosen old deposits such as rust and dirt which must be eliminated to prevent clogging the fuel passages. Once this is accomplished, no further action is necessary. Finally, the alcohol must contain a denaturant that is not too expensive, will not cause separation of components at lower temperatures, and will not form gum.

In the injection method of using alcohol as motor fuel, an alcohol-water mixture is fed automatically into the manifold by means of a small auxiliary carburetor when the engine is working near or at full load, that is, during hill climbing or acceleration. In ordinary driving on level or nearly level roads at even speed, most passenger cars can operate knock-free with gasoline of 50-octane number instead of a regular gasoline having a Research method octane number of 80.

Experiments conducted under a Research and Marketing Act project showed that in average city and country driving of a car equipped with alcohol-water injection, about 1 gallon of alcohol-water mixture is needed for every 50 to 65 gallons of low-octane gasoline used. For trucks the proportion of alcohol is higher and depends on the type of service. Alcohol-water injection for getting more power when needed has the advantage of economy and could put alcohol on a more competitive basis. This would be particularly true in engines having higher compression ratios than are commonly used. In such engines present-day regular or premium gasoline could be used in combination with alcohol-water injection to supply a superoctane fuel without having to manufacture it. The manufacture of superoctane gasoline is extremely costly, and more crude oil is required per unit volume. High-compression engines are more economical, as shown by the fact that the thermal efficiencies at 6 : 1 and 10 : 1 compression ratios are 36 percent and 43 percent, respectively—a gain of about 20 percent. Because of the tendency toward higher-compression-ratio engines in new cars, which necessitates higher octane values, the fuel problem is becoming more complex. Refineries desiring to compete would have to invest many billions of dollars for the installation of expensive equipment to produce superoctane gasoline with lower yields of gasoline from a given quantity of crude petroleum. Smaller refineries might not be able to afford the cost of conversion. Hence the use of alcohol for motor fuel should prove desirable for preventing the waste of much valuable material as well as by making such costly investments unnecessary.

Good Plasticizers Made Cheaply From Lactic Acid

Plasticizers or softeners must be used in many products made from synthetic resins and plastics which require about 200,000,000 pounds of plasticizer annually. In the report for 1947 it was stated that several plasticizers suitable for use with vinyl resins had been made from lactic acid. Since lactic acid can be produced easily and cheaply from many agricultural carbohydrate byproducts and wastes, its use in making plasticizers might furnish a large outlet for such materials. More and better plasticizers have been made recently, and, equally important, a simpler and cheaper process has been developed for their manufacture.

About 100 high-boiling esters of lactic acid were synthesized and evaluated as plasticizers by several screening tests. One of the most efficient plasticizers for vinyl chloride copolymer (95-percent chloride)

was butyl lactate adipate, a plasticizer that can be prepared from butyl alcohol, lactic acid, and adipic acid. These raw materials are readily available at low cost.

To produce this plasticizer, butyl lactate, made from butyl alcohol and lactic acid, is treated directly with adipic acid. This avoids the use of the more expensive and corrosive acid chloride commonly used in laboratory preparations. Although the use of adipic acid results in the production of a mixture of several products, it was found that this mixture, which contains butyl lactate adipate as the main component, was equal or superior to the pure ester as a plasticizer. Furthermore the mixture was obtained in high yield. It was also shown that the crude, undistilled mixture was as effective as the distilled material. Since the color of the crude product is light, making it unobjectionable for many purposes, most of the product could be used commercially in the crude form.

Because of the availability and low cost of the raw materials and the effectiveness of the products, several manufacturers are interested in plasticizers made from lactic acid and are considering their commercial production.

SUGAR-BEARING PLANTS AND SUGARS

Sugar Mills Helped in Solving Problems of Processing Machine-Cut Cane

Mechanical harvesting of sugarcane has brought to the cane-sugar industry a number of new processing problems that require solution. In the 5 years since the beginning of widespread use of machines for cutting sugarcane, yield of sugar per ton of cane has dropped from 175 to 150 pounds. The presence of excessive trash—various types of leafy material and immature joints of the stalk—is considered the major cause of the new difficulties. Sometimes the dissolved solids in juice from such trash consist of only ⅕ sugar and ⅘ nonsugar constituents.

Investigations were conducted by the Agricultural Chemical Research Division to aid sugar producers in coping with this situation. In three lines of recent work valuable contributions were made. As a result of the first investigation, data were obtained and published which indicated that the side leaves on the cane caused the most trouble. Improper topping during machine-cutting, i. e., trimming the cane too near the top, was found to introduce an unusual amount of leafy material that has a very low sugar content. This information was disclosed at a meeting of the contact committee of the Sugar Cane League in Thibodaux, La., last March, and was welcomed as a definite step toward solving the processing problems associated with mechanically harvested cane.

Since grinding must be delayed sometimes, a second investigation was made to determine the conditions under which cane can be stored longest without deterioration. When the standing cane is burned over to destroy excessive leafy material before being harvested mechanically, the dead, burned tissue is susceptible to micro-organisms that thrive in dampness. It was shown that burned cane that is mechanically harvested, in contrast with hand-cut cane, must be kept thor-

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oughly dry if it is to be held any length of time before grinding.

A third investigation was concerned with the juice-clarification step in sugar production—the step in which impurities are precipitated by adding lime and heating the juice. In periods of wet weather more field mud, as well as trash, is brought in with the cane. Juice from cane handled under these conditions produces a precipitate that is too voluminous and does not settle readily. This slows down the process. Treatment of such juice with oystershell flour as well as lime was found to be advantageous and was recommended to sugarcane processors as an aid to good clarification.

So far, about 20 factories have tried the treatment. Several reported that oystershell flour proved so beneficial during liming that plants could be operated at reasonable capacity even during rainy periods. One processor reported that the treatment permitted such good juice clarification that his plant ran up to 80 percent of its full capacity during wet weather, and with cane that would otherwise have reduced operations to about 50 percent of capacity.

This brief statement gives some idea of the problems that require solution if full benefits are to be derived from the use of mechanical harvesting in sugarcane farming.

New Sugar, Isomaltose, Isolated From Cornstarch

This year a goal long sought by carbohydrate chemists and practical users of starch and starch-rich grain has been attained. For the first time, the theoretically existing sugar isomaltose, or 6-[*alpha-d*-glucopyranosyl]-*d*-glucose, has been isolated in crystalline form from starch by chemists working at the Northern Regional Research Laboratory. In research under a cooperative agreement with the Corn Industries Research Foundation, a method has also been perfected whereby isomaltose can be prepared in quantity for further study. Yields of around 5 percent have been obtained from waxy or glutinous cornstarch and from the nonlinear, or amylopectin, fraction of ordinary cornstarch.

In the common cereal grains and cereal starches the dextrose units making up the large starch molecules are combined by two types of bonds. About 96 percent of the bonds or linkages are of the alpha-1,4-type which exists in maltose, the simplest disaccharide having this part of the starch structure. The remaining 4 percent of the linkages are of the alpha-1,6-type, which corresponds to the disaccharide isomaltose. This sugar was isolated from starch only recently, although the existence of an anomalous alpha-1,6-chemical linkage in starch has long been known. The alpha-1,6-linkage has been considered more difficult to break than the normal alpha-1,4-linkage, both by acids and enzymes. The greater energy required to break the alpha-1,6-linkage is partly responsible for suboptimal yields and the necessity of using drastic conditions of hydrolysis in present starch-conversion processes.

The isolation from starch of a disaccharide composed of two glucose residues linked together in the alpha-1,6-positions places in the hands of the chemist the simplest sugar containing this type of bond. The availability of isomaltose makes it possible for chemists and fermentologists to determine with exactness the properties and behavior of this sugar. Experiments can now be carried out to show the most practicable conditions for the conversion of this disaccharide into monosac-

charide by acid and enzymic hydrolysis. This is of much importance in the industrial conversion of starch into dextrose sugar, glucose sirups, and other sweetening agents. Studies of the behavior of isomaltose toward the amylaceous enzymes from barley malt, as well as those from mold and bacterial preparations, are now possible. Optimum conditions can be ascertained for the cleavage of the alpha-1,6-bonds by enzymes. In addition, studies can be directed toward the production of enzyme preparations having increased or specific activity in the cleavage of alpha-1,6-bonds in starch.

New starch products and improved processes for the industrial conversion of starch and cereal grains are results to be reasonably expected from this isolation of isomaltose.

Crystalline Compounds of Sugar and Water Discovered

Seven crystalline compounds of sucrose (table sugar) and water, known chemically as sucrose hydrates, have been discovered at the Western Regional Research Laboratory during an investigation of the behavior of sugar solutions at subfreezing temperatures in relation to the freezing preservation of fruit. No solid sucrose hydrates had been reported prior to this investigation.

These hydrates usually grow in spherulitic aggregates of fine needles in a cauliflowerlike formation. Hydrate I, however, can be obtained as single monoclinic crystals which can, in time, be grown to large dimensions (1 inch or more in length). These crystals are commonly twinned, which gives them a pseudo-orthorhombic appearance. All seven hydrates appear to be metastable above 3° C. Above this temperature they decompose into anhydrous sucrose and water; below it they change into the more stable hydrates so slowly that each can be handled as a stable phase in the absence of the others. The compositions of sucrose hydrate-ice mixtures having minimum melting points (eutectics) lie within the narrow range of 46.5 percent sucrose for hydrate VII, with melting point of −6° C. and 58.0 percent sucrose for hydrate I, with melting point of −10.5° C. The sucrose hydrate-ice eutectics are hard dry solids and crystallize readily. This is in marked contrast to the anhydrous sucrose-ice eutectic, which could not be obtained in numerous attempts. It appears very unlikely that the anhydrous sucrose-ice eutectic will form in sucrose solutions at low temperatures.

Hydrate II, the one that is usually found in frozen fruit stored below −11° C. in sirup containing less than 70 percent sucrose, was found to contain 3.5 molecules of water for each molecule of sucrose. Hydrate I, which is usually found when the sucrose concentration is over 70 percent, contains 2.5 molecules of water for each molecule of sucrose. The spontaneous crystallization of these hydrates in frozen fruit is facilitated by frequent fluctuations in storage temperature as well as by the high sucrose concentrations that result from surface evaporation. The effect of increased concentration of sucrose caused by the separation of ice as storage temperature is lowered is compensated to some extent by the resulting increased viscosity.

The unsightly moldlike growth that is sometimes found on frozen food packed in sugar or sirup has been identified as a harmless crystallization of sucrose hydrates. Many representative substances such as corn sirups, sugars, dextrins, starches, pectin, sodium alginate, gly-

cerine, propylene glycol, fruit acids, gelatin, agar, salts, and glycine have been investigated to determine whether they would inhibit the growth of sucrose hydrate. These additives were tried in proportions ranging up to 50 percent of the total solids and 35 percent of the total solutions. None of the compounds tried was able to prevent sucrose hydrate growth at −10° F. (−24° C.) in solutions containing a small amount of added hydrate crystal seed, although several sugars (glucose, fructose, invert sugar, and maltose) in high proportions appeared to retard hydrate growth. Unfortunately, these sugars also form hydrates which may be just as undesirable as the sucrose hydrates.

Further work is being carried out on other sugars, particularly on fructose, about which very little information has been published. A crystalline dihydrate of fructose was recently discovered, and its properties are now under investigation.

New Uses Sought for Maple Products

Work recently started at the Eastern Regional Research Laboratory under an RMA project is expected to lead to improved methods of producing maple sirup, better quality and greater uniformity of sirup, and the development of new products and uses. Efforts have been made to determine the source and identity of the maple flavor, the only ingredient of edible maple products that is of economic importance. A complete understanding of this characteristic flavor is necessary before any significant changes in either production or utilization methods can be made. In the course of this work an experimental maple sirup that is almost entirely free of both maple flavor and the associated color was made by low-temperature vacuum evaporation of sugar-maple sap. This sirup is now being used as the basic material in flavor studies. The production of such a sirup, together with other substantiating evidence, strongly indicates that the flavor and color of maple sirup are not preexistent in the sap, but are developed by conventional processing after the sap leaves the tree. Investigations are now under way to identify the constituents of normal maple sap that produce the characteristic maple flavor when they are brought together under the proper conditions of temperature and heating time.

The Bureau is cooperating with the Fruit and Vegetable Branch of Production and Marketing Administration in the development of a color comparator and permanent color standards for maple sirup.

MISCELLANEOUS VEGETABLE PRODUCTS

Sensitive Laboratory Test Aids the Cucumber-Pickle Industry

Plant operators in the cucumber-pickle industry in certain seasons suffer large financial losses due to softening of their fermenting cucumbers during storage. Unusually heavy losses in 1947 and 1948—amounting to hundreds of thousands of dollars at plants in the southeastern brining area alone—led processors to seek assistance of the Agricultural Chemical Research Division in making a bacteriological investigation of the nature and causes of deterioration of their stored brine-stock.

The cause for the lack of firmness was found to be enzymatic in nature. A laboratory test was developed which detects the first appearance of enzymes in brine, and by its use the particular enzymes causing the trouble were identified. The success of the new test (based on the change in viscosity of a pectin solution upon adding brine from the curing solution) was established by examination of 239 commercial vat brines in brining areas of nine States, including three northern areas.

The early detection of the softening (pectin-splitting) enzymes in cucumber brines would be invaluable to plant operators in forecasting possible softening of brine-stock and in applying remedies to prevent it. For instances, brine-stock showing the first signs of the enzymes would be immediately processed into pickles; for if left in the vat the stock would make only inferior products. Application of the test by cucumber-pickling plants would thus substantially reduce the economic losses long suffered in the industry because of softening during storage.

A second development of this investigation was the isolation of a new strain of undesirable yeast, *Torulopsis caroliniana*, which is associated with gas formation and therefore another cause of deterioration of cucumber brine-stock and of economic losses to picklers. The conditions for preventing growth of this organism are being determined.

Production and Use of Leaf Meals From Vegetable Wastes Studied on Commercial Scale

Since work at the Eastern Laboratory on the utilization of vegetable wastes had progressed to the stage where large-scale drying of the material was indicated, a research contract was arranged with a large vegetable growing and packing company in New Jersey under an RMA project. Some 50,000 tons of fresh vegetable waste, mostly leaves, are available to this company each year over a 6-month period. Therefore tons, rather than pounds, of leaf meals will be produced, and their feeding value and marketability will be determined from large-scale experiments. Beet and lima bean leaves will receive special attention, although spinach, pea vines, carrot tops, and broccoli leaves will probably come into the picture. A building, 50 by 82 feet, was erected to house the equipment, which includes hammer mills, a dewatering press, an ensilage cutter, three rotating cylindrical driers with oil burners, cyclone separators, a vibrating screen, a bagger, and necessary conveyors. The equipment is arranged in a flexible manner so that various systems of handling can be tried on the various types of leaves. Carotene and protein will be determined in the company's laboratory.

Preliminary laboratory and pilot-plant experiments at the Eastern Laboratory had established the background for this project. A survey showed that the leaf blades were high in protein, carotene, and riboflavin, while the stems and midribs were relatively low in them. A mechanical method was devised for separating the blade portion from stems, after flash drying, with an efficiency of about 95 percent. Conditions of drying and types of driers were studied, especially with regard to sparing the carotene. The fate of carotene under various conditions of storage was determined. Detailed studies of other constituents—chlorophyll, phytol, sterols, tocopherol, glycerides—

were made. Through cooperative arrangements, leaf meals of various sorts were fed to poultry at the Delaware Agricultural Experiment Station to determine relative nutritional value.

Carotene (provitamin A) is the most unstable of the nutrient constituents in leaves. It begins to disappear when cut plants are left in the field to wilt and decomposes rapidly if the leaves are badly bruised. Prompt drying is the remedy in both cases. After the leaves are dried and bagged, the temperature of storage is important. Most meals lose 30 to 50 percent of their carotene at 75° F. in 3 months, but only half as much at 40°.

With the larger amounts of leaf meals produced under this project, feeding trials on larger animals can be made, compatibility in mixed feeds determined, and behavior of the carotene in storage studied.

FRUITS AND FRUIT PRODUCTS

Commercial Production of Concentrated Orange Juice Increases Rapidly

A process for the production of a 4-fold-concentrated frozen orange juice which was developed at the Winter Haven, Fla., laboratory of the Agricultural Chemical Research Division in cooperation with the Florida Citrus Commission, was previously reported. Recently this process has received Nation-wide publicity and has been applied by a considerable number of processors. Last season about 9 million gallons of the concentrate were put on the market. The estimate for 1949–50 production is 20 million gallons. Eleven plants are now in operation in Florida, and more are expected to be in operation soon. The process has had a beneficial effect on the market price of oranges and is providing the public with a product of excellent quality. Its economic importance to citrus fruit growers cannot be overemphasized. The expanded production of fourfold concentrate should have a stabilizing influence on the whole orange industry.

Frozen Citrus Purees Produced Commercially

Frozen orange and lemon purees developed through research in the Bureau's Laboratory of Fruit and Vegetable Chemistry in Los Angeles, Calif., are now being produced commercially and sold to bakeries, ice cream manufacturers, and other food processors throughout the United States. The freezing preservation of citrus fruit purees has proved to be a highly efficient and economical process for the preparation of fruit bases having original flavor, color, and nutritive value. These fruit bases can be kept in good condition by storing at 0° F. for more than a year with little, if any, loss of the original flavor and vitamin C content.

The pureeing of citrus fruits offers three distinct advantages in the preservation of these products. First, the sugar added to the puree dissolves in the juice and comes in intimate contact with the fruit cells, which thus become surrounded by a protective coating of sirup. Second, the product thus prepared forms a solid mass on freezing, which helps to exclude air. Third, the frozen puree can be defrosted to permit removal of some for use and then be refrozen without injury to the color or flavor, provided it is not exposed to the air for a long

period and the temperature of the product is not allowed to rise too high.

If a plant that processes deciduous fruit can include the freezing preservation of citrus purees its operating season can be extended over a longer period. For example, the plants that freeze deciduous fruits in southern California would ordinarily shut down after the last deciduous fruit crops have been harvested in late summer, but they could continue their operations till November by freezing purees of California Valencia oranges and even later in the season by using Arizona Valencia and Arizona "Sweet" oranges. Lemons can be processed in almost any month of the year.

The pureeing of citrus fruits for freezing preservation also has a distinct advantage for the grower, since fruit having good color and flavor can be utilized regardless of its shape, size, or superficial blemishes that make it unfit for the fresh-fruit market. This process can aid in profitable utilization of a larger percentage of the citrus crop than has been possible heretofore. Large-scale production of frozen citrus purees has just begun and so far has been confined to California. However it is anticipated that the process will expand to other citrus-producing areas.

Both orange and lemon purees have been successfully used in the commercial preparation of milk sherbets and water ices. However, taste tests have indicated that the milk sherbets, which contain about 2.5 percent butterfat, have more pleasing flavor and smoother texture than the water ices. Minute pieces of the orange and lemon peel are detectable in both sherbets and ices made from the citrus purees; these provide visual evidence to the consumer that the products are flavored with natural fruits. Besides being useful for making frozen desserts such as sherbets and ices, they can be used to make flavorful cakes, pies, jams, marmalades, and beverages like lemonade.

Of particular significance to California citrus growers is the possibility that purees can be produced successfully from navel oranges. This type of fruit, which constitutes more than one-third of the California orange production, has previously not been suitable for processing because the juice has a tendency to become bitter. Also some difficulty has been experienced in trying to jell navel orange purees. Work is being continued to solve these problems.

Study Made of Flavor Changes in Western Citrus Juices

Several years ago the Arizona-California Desert Grapefruit Industry Boards initiated a cooperative research program at the Bureau's Laboratory of Fruit and Vegetable Chemistry in Los Angeles, Calif., on the isolation and identification of the volatile flavoring constituents of fresh and canned grapefruit juices. The purpose was to determine what chemical changes take place in the volatile constituents to cause off-flavors and odors in the processed juice. The canned juice of western-grown grapefruit is not acceptable to some persons because the flavor is not always characteristic of fresh juice. It appears that foreign flavors are formed through the break-down of certain naturally occurring constituents.

Under a Research and Marketing Act project this Bureau has undertaken the chemical identification of the volatile constituents of juice from western-grown citrus fruits and proposes to learn which ones

break down on processing and storage to form the objectionable off-flavors. By the use of more closely controlled processing methods and more desirable storage temperatures, it may be possible to prevent some of these chemical changes and produce canned citrus juice of enhanced quality. During the past fiscal year this work was transferred to a new laboratory at Pasadena and expanded to include a study of the volatile constituents of fresh and canned orange juice as well as grapefruit juice.

In general, the method used for isolating the volatile constituents consists of distilling large quantities (2,500–3,000 gallons) of the juice under carefully controlled conditions of temperature and partial vacuum until 50 percent of the juice has distilled over. Cold traps ranging in temperature from 0° C. to the temperature of liquid air are required to condense the most volatile fractions under the low pressure. Two 3,000-gallon lots of fresh juice, one from oranges and the other from grapefruit, were distilled and fractionated, along with two 2,500-gallon lots of the corresponding newly canned juices. Two additional lots of the canned citrus fruit juices are being stored at room temperature, and after 1½ to 2 years they will be similarly distilled, fractionated, and analyzed for comparison with fresh and newly canned juices.

Identification of the various volatile fractions (mainly essential oils) is made by accepted and newly developed chemical and physical methods. A compound having the tentative formula $C_6H_{10}O$ was identified in the fresh and canned grapefruit juices, as well as a C_{10} compound. Furfuraldehyde was identified in the volatile-oil fraction from the canned grapefruit juice, but none was found in the corresponding fraction from the fresh juice. Preliminary storage tests indicated that the furfuraldehyde content of canned grapefruit juice increases with an increase in off-flavor. Traces of this compound were found in the fresh orange juice, but not in the fresh grapefruit juice. Ethyl alcohol and traces of methyl alcohol were found in all the juices examined. Both acetaldehyde and acetone were found, the former in large amounts. The liquid-nitrogen traps were found to contain carbon dioxide and hydrogen sulfide.

Sulfur Compounds in Citrus Juice May Affect Flavor

The odors and flavors of plant materials are in many cases related to the sulfur compounds they contain. Although most of the known sulfur compounds possess strong and unpleasant odors and tastes, some of them are fruity and aromatic. Certain pleasant-smelling sulfur compounds may contribute to the aroma and flavor of fruit, but they are liable to decomposition by bacteria and enzymes, with the formation of very bad-smelling products, such as hydrogen sulfide and the mercaptans, which contain sulfur in the reduced form known as sulfhydryl. Thus the investigation of sulfur compounds, and particularly of sulfhydryl compounds in citrus fruit, has a direct connection with the question of flavor and development of off-flavors in citrus juice.

The Enzyme Research Division, in work on the improvement of citrus products under an RMA project, found that sulfur compounds of the sulfhydryl class are present in the juice of ripe oranges, both navels and Valencias, to the extent of about 50 parts per million. It accomplished the separation of two sulfur compounds from the other constituents

of the juice by means of precipitation with salts of mercury. The mercury precipitate was then decomposed to liberate the original sulfur compounds, which were eventually isolated as crystalline derivatives. Two distinct compounds were thus obtained, separable because of their different solubilities. They were then identified by their crystalline form, optical rotation, and chemical analysis.

These two substances represented at least four-fifths of all the sulfhydryl sulfur compounds present in the original juice. Their identification is not entirely completed, but it is known that the two substances are either cysteine and glutathione or compounds very closely related to them. Cysteine is an amino acid, and glutathione a tripeptide (gamma-glutamyl-cysteinyl-glycine). Both substances contain nitrogen as well as sulfur; the latter has previously been found in other natural products.

The sulfur compounds were isolated from six crates of California navel oranges picked fairly early in the season. However, after the identity of the two sulfur compounds was learned, it became possible to demonstrate their presence and estimate their relative amounts in several other lots of orange juice, without actually isolating the substances themselves. It was observed that there was little variation between juices from navel and Valencia oranges and that the quantity of these substances present did not vary greatly with the degree of ripeness of the fruit. In each case about 80 percent of the total sulfhydryl sulfur could be precipitated with mercury, and glutathione was found to be by far the more abundant of the two compounds, there being about 12 parts by weight of glutathione to 1 of cysteine.

Glutathione and cysteine have a faintly aromatic smell and a slightly burnt taste, but these properties are not so pronounced as to make either of them a flavoring material. Both are easily metabolized by micro-organisms and also easily acted upon by bacterial enzymes to cause the liberation of hydrogen sulfide, a very bad-smelling substance. Hydrogen sulfide is also known to be the cause of the brown spots that appear on the interior surfaces of the cans. Evidently the decomposition of these sulfur compounds can produce off-flavors, and the possibility of such decomposition occurring in processed orange juice can be studied, since the substances in question are now known. The mechanism of this reaction must, however, be the subject of further study.

Two-Stage Process Makes Better Grape Essence

Treatment of commercial pasteurized grape juice by the Eastern Regional Research Laboratory's apple-essence-recovery process, modified by vaporizing 20 percent of the juice instead of 8 to 10 percent, gives a fairly satisfactory grape essence. However, a stronger essence of still better quality can be produced by adding to the primary essence a secondary one made from the distillate obtained in concentrating the stripped juice to a sirup after it has been depectinized. The equipment originally designed for obtaining flavor essence from apple juice can be used for both of these operations.

The complete two-stage process was developed with commercial pasteurized Concord grape juice. The primary essence was made by very rapidly vaporizing 20 percent of the juice, the vapor being conducted to a packed column in which the volatile constituents were further concentrated. Condensation of this concentrated vapor gave

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1 gallon of essence per 100 gallons of juice used, i. e., a "hundredfold" essence. This had a highly aromatic odor typical of Concord grape juice. The stripped juice resulting from the 20-percent vaporization was depectinized with a pectinase enzyme, after which it was filtered to remove precipitated argol and coagulated pectic acid and passed directly into a vacuum evaporator provided with a surface condenser. To minimize the possibility of heat damage to the juice, the temperature of the batch during evaporation was kept down to 100°–110° F.; this required a vacuum of about 28 inches. The juice was evaporated as rapidly as possible to a concentrate of sirupy consistency (solids content 65°–70° Brix) which was self-preserving against fermentation. The distillate obtained during the concentration amounted to about 60 gallons per 100 gallons of original juice. It had the characteristic aroma of Concord grape juice which was found to be recoverable in the form of a secondary essence by vaporizing 20 percent of the 60 gallons of distillate and concentrating and condensing the more volatile constituents of the vapor as in making the first essence. In this way 0.6 gallon of secondary essence per 100 gallons of original juice was obtained.

A reconstituted grape juice was made by combining the primary and secondary essences with the juice concentrate and adding enough water to make 100 gallons, thus forming a juice of almost the same solids content as the original, only the pectic acid and argol having been removed. This was found to be identical with the original commercial grape juice in odor and taste.

Tests will be made to discover whether cold storage is necessary for preserving the "full-flavor concentrate" made by adding the two essences to the bland concentrate. This full-flavored concentrate may fit in well with the current vogue of packing concentrated fruit juices, since it offers a great saving in the cost of distribution. Another advantage of this process is that precipitation of argol from the freshly pasteurized juice, similar to what is customarily accomplished by long storage of the juice, is obtained quickly during the evaporation of juice to sirup.

Pear-Cannery Waste May Yield Feed Yeast

Pilot-plant studies on a process for the production of feed yeast from pear-waste juice were continued at the plant of a collaborating canning company in Olympia, Wash. The process, as developed in the pilot plant by the Western Regional Research Laboratory, comprises continuous, constant-volume, submerged-culture propagation of torula yeast. It appears to have two distinct advantages over most other continuous yeast-propagation processes. First the operation is under automatic instrument control which constantly maintains nearly the optimum conditions that cannot be maintained easily with manual control. The second advantage is more effective air dispersion, which results from use of an improved mechanical air-dispersing system.

The optimum production capacity of the pilot-plant propagator exceeded that of laboratory propagators of similar type, and the reproduction rate was higher than has previously been reported for other experimental or commercial propagations of the same organism. Further, the concentration of sugar in the feed wort was consistently

much higher than levels reported by other investigators to be limiting to yeast growth.

The practical importance of these findings lies in the large production that can be obtained from a small yeast propagator. The high reproduction rate results in a short average retention time for the yeast cells, and the high level of sugar in the wort permits maintenance of a high yeast concentration in the propagator. Results of this work have stimulated considerable interest on the part of commercial feed-yeast producers.

One of the major problems has been an economical separation of pear waste into fractions suitable for further processing, namely, a clear juice and a fiber fraction dry enough to be handled in a rotary drier. During the year laboratory studies disclosed the conditions under which separation of juice and fiber fractions of untreated waste is likely to be most feasible. The results are being used by a commercial firm in studies under a Research and Marketing Act contract on the design of a continuous press. The contractor has designed and tested workable press elements that appear to be sound and suitable for a multiple-unit, pilot-plant press. Practical tests of such a press are in prospect.

Laboratory studies have been made of procedures for chemically treating fruit wastes, particularly pear waste, to facilitate separation of a juice fraction. The results indicated that after preliminary chemical treatment economical separation may be possible in conventional types of commercial pressing equipment.

Cooperative tests with a large group of pear canners were planned for the 1949 season. A successful outcome would be an important step toward alleviation of a critical waste-disposal problem now facing the fruit canners in many processing areas.

Sirup Filling Improves Frozen Apple Slices

The usual methods of pretreating apple slices for preservation by freezing have been designed chiefly for the prevention of enzymatic browning after freezing and thawing. While they are more or less satisfactory for that purpose, they are usually objectionable in other ways. For example, steam blanching causes a loss of soluble solids, excessive softening in some varieties, and flavor changes. The use of sulfites in the ordinary manner usually imparts a bad flavor because of too much chemical. Pretreatment of slices with either sulfites or ascorbic acid according to common procedures may lead to poor texture in the thawed product, manifested by heavy leakage, "drip", or to poor appearance and excessive softness after cooking.

The "sirup-filling" treatment, as developed by the Western Regional Research Laboratory, is intended to prevent the browning of apple slices after freezing and thawing while at the same time producing a product with better flavor, texture, and drip characteristics than would result from the usual procedures. It comprises removal of tissue gases from the slices by the use of a vacuum, immediately followed by partial filling of the voids with sugar sirup containing a small quantity of antioxidant. Successful application depends upon careful attention to various factors, including concentration of sirup, length of exposure of the slices to vacuum, duration of contact of slices with sirup, and concentration of the antioxidant in the sirup.

Sirup-filled apple slices (processed by vacuum impregnation with 60-percent sucrose sirup containing 0.3 percent of ascorbic acid) were prepared from Winesap, Gravenstein, Yellow Newtown, Delicious, Red Delicious, Jonathan, Rome Beauty, Cortland, and McIntosh apples. After being frozen and stored about 6 months, these were evaluated in the making of pies to determine the relative effectiveness of the special enzyme inactivating procedure as compared to control samples processed by usual methods.

The greatest over-all improvement due to the sirup-filling treatment was observed in soft varieties such as Gravenstein, McIntosh, Cortland, and Rome Beauty. The baked fruit from sulfilted or steam-blanched slices prepared from these varieties was essentially a mushy sauce, lacking in natural flavor, whereas the sirup-filled slices, although soft, retained their normal shape and appearance as well as most of their natural fresh flavor. Textural differences between treatments were less pronounced in the Winesap, Yellow Newtown, and Jonathan samples; however, for all varieties tested, the flavor of the sirup-filled samples was eminently superior to that of the samples treated otherwise. Sirup filling effected a marked improvement in appearance and texture of baked slices of both Delicious and Red Delicious apples as compared to sulfited or steam blanched samples.

Estimates based on the procedures used in experimental studies indicated that costs of a sirup-filling process would be higher than those of conventional processes. It thus appears that the competitive position of sirup-filled slices would depend largely on improved quality. This would favor application of this method to soft varieties where the greatest quality advantage could be derived.

New Facts Sought on Enzymatic Browning of Fruits

The problem of enzymatic browning of peeled fruits such as apples, apricots, and peaches is an old one and is particularly significant in freezing procedures where avoidance of heat leaves the enzyme systems largely intact. Available evidence indicates that the browning process involves enzymatic oxidation of phenolic substances, classified as tannins. However, there is a dearth of information on the nature of these substances, their concentration in various fruits, and the steps involved in their conversion to brownish pigments. Moreover, there is lack of information on the enzyme systems involved in the oxidations. Further advances in control of enzymatic browning of frozen or otherwise processed fruits depend in large measure upon greatly increased knowledge of the kind mentioned.

Previous reports described a procedure for estimating the tendency of fruits toward oxidative discoloration. It involves quantitative determination of enzyme (catecholase) activity and of phenolic substances. Application of this procedure to peaches and analysis of the results revealed that (1) only a fraction of the total phenolic substances is enzymatically oxidizable, (2) the rate and extent of browning can be ascribed only to this fraction and to the activity of the enzyme system, and (3) concentrations of both enzyme and substrate vary greatly with variety, locality, and season. Samples of Elberta peaches harvested in 1948 from three different growing areas

showed marked variations in tannin content with climate. Fruits grown in a warm and sunny area were lower in tannin than those grown in comparatively cool, cloudy areas.

Two fundamental investigations were started on the problem of enzymatic browning. One deals with the purification and characterization of polyphenolase from apples, the other with the mechanisms of the enzymatic oxidation reaction. A partial purification of the enzyme has been accomplished through extraction of the apple tissue with a sodium carbonate solution and precipitation of the enzyme with acetone at about −20° C. It is noteworthy that catalytic activity is not lost through conversion of the enzyme to the insoluble form. Work on further purification of the enzyme is in progress.

Experiments on the partly purified enzyme showed that it differs from a previously described polyphenolase isolated from mushrooms. In contrast with the reported behavior of the latter, the apple polyphenolase is not irreversibly inactivated during oxidation of catechol. These two polyphenolases differ in their behavior toward hydroquinone which acts as a protective agent toward the mushroom enzyme but inhibits apple polyphenolase.

The mechanism of the enzymatic oxidation is being investigated through use of model systems. Various substituted catechols have been synthesized for the purpose. The stoichiometry and kinetics of oxidation of these substances will be measured as a means of arriving at an understanding of the browning process.

Peach and Apricot Canners Aided in Pacific Northwest

The freestone-peach canning industry of the Pacific Northwest has made rapid, continuous growth since its beginning about 1935, and the current annual production of over 1 million cases appears to be limited only by the availability of fresh fruit. The high-quality products obtainable from freestone peaches grown in this area makes this crop one of the most promising for future expansion. This high quality is due in considerable part to the development of new and improved methods for handling and processing. Contributions made to the technology of freestone-peach canning by the Bureau's Fruit and Vegetable Products Laboratory at Pullman, Wash., during the past 12 years have had an important influence on the development of this industry and will prove to be of increasing value as the industry expands. Through studies in cooperation with Washington's agricultural experiment stations, basic information has been contributed on the suitability of different varieties for canning and on harvesting, ripening, storage, and processing methods.

In 1944 information was released about canning studies on 46 peach varieties, of which Elberta, Early Elberta, and Gold Medal were found most desirable for canning. The use of other varieties was also suggested to permit extension of the canning season, even though they required special attention in harvesting to assure canned products of high quality. Information on canning characteristics of different varieties has served the industry effectively in its program of expansion and quality improvement. Data on size, color, time required to ripen, wilting losses, yield, and quality of canned fruit have been ob-

tained and used in the development of practical recommendations to guide the industry in harvesting operations. The industry has benefited especially from information that enables operators to reduce losses due to bruising and to immature color and flavor in the canned product.

Following repeated laboratory tests under a wide variety of conditions, it was concluded that the best ripening conditions for freestone peaches are closely approximated, under commercial conditions, when large blocks of fruit are stored in open lots with protection from direct sunlight, or in well-ventilated common storage warehouses. Thus expensive ripening facilities were shown to be unnecessary. It was found that freestone peaches of optimum harvest maturity can be stored for as long as 4 weeks, provided storage temperature is maintained at 31°–32° F. Lengthening of the processing season is thus made practical.

Canning studies, reported in 1946, showed that discoloration after peeling was retarded by increasing the steaming time to 90 seconds. Discoloration in the canned product was also reduced by decreasing the oxygen in the container and increasing the exhaust time. Increased exhaust temperatures also prevented "chalky" areas in the product. Reports have been made of preliminary studies on equipment for grading freestone peaches. Successful mechanical grading would reduce processing costs and improve quality.

Information obtained in the course of these studies was recently summarized in a 40-page circular to increase its usefulness to the industry, and assistance was given to the Washington State College Agricultural Extension Service, the Washington State Fruit Commission, and the Northwest Canners Association in a program to acquaint growers and processors more fully with the availability of this information.

Results of a three-season cooperative study on canning Washington-grown apricots have provided the first technical information available on the processing of this increasingly important Washington fruit. Expanded processing outlets for apricots in this area are necessary to stabilize the fresh-fruit market and to avoid loss of fruit in seasons of high production. The relative canning quality of 17 varieties was determined. Of these, Blenheim, Tilton, and Royal were considered most suitable for canning. Wenatchee Moorpark, the principal commercial variety in Washington, was unsuitable for canning because of its undesirably soft texture and the poor appearance caused by sloughing of the skins. This variety could be used, however, to produce a canned nectar of excellent flavor, bright deep-orange color, and desirably viscous body which would probably have considerable demand.

Improved methods were developed for handling the Wenatchee Moorpark apricot to overcome its uneven ripening characteristics. This variety developed the deepest color and best flavor when ripened at 75° F. with confinement of the gaseous emanations. Ventilation of the fruit during ripening at this temperature resulted in slow ripening and poor color. At 95° F. gaseous emanations from the fruit appeared to inhibit ripening and resulted in poor flavor.

MISCELLANEOUS FOOD PRODUCTS AND PROCESSES

New Food-Freezing Method Studied

Indirect immersion in refrigerated brine has received intensive study during the year as a new freezing method for foods. The results emphasized possible advantages over present commercial methods—air-blast and plate freezing—and bring the method closer to commercial application by removing some of the objections and supplying essential design data. Corrosion of cans in storage after immersion in brine has been cited as a possible objection to the method; however, cans that are washed thoroughly with water sprays will give no trouble.

In this method the material to be frozen is packed in hermetically sealed cans which are immersed in refrigerated calcium chloride brine until frozen. One objection in the past has been the supposed danger from growth of food-poisoning organisms, such as *Clostridium botulinum*, especially in nonacid foods, in the hermetic container required to protect the unsterilized food from the liquid refrigerating medium. In the past 2 years, however, bacteriological investigations undertaken in cooperation with the G. W. Hooper Foundation for Medical Research showed that frozen vegetables can be packed hermetically without danger, since normally occurring nontoxic strains of bacteria will prevent the growth of the *Cl. botulinum*. This information has opened the way to the development of the indirect immersion freezing method.

Freezing rate data were obtained for liquid food products having relatively high thermal conductivities, for example fruit juices and purees, and also for loose aggregates having low thermal conductivities, such as drained peas, diced carrots, sliced apples, and brussels sprouts. The effects of brine temperature, brine velocity, container size, and manner of exposure to the brine stream were determined, as well as the effect of can rotation during freezing.

Rates of freezing by indirect immersion were found to be several times as fast as those obtained in conventional air-blast package-freezing systems and appreciably faster than those obtained in the high-cost plate freezers. Rotation of cans resulted in further large reduction in freezing time for liquid products but had no material effect on nonliquids. Container position and velocity of refrigerant were not found to be critical. The data obtained will be used in the development of improved designs for immersion freezing equipment.

General adoption of the indirect immersion freezing method is contingent upon product distribution factors that have not yet been adequately studied. The possible operating advantages of indirect immersion freezing are such that it may find wider application in the freezing of products in small cans for the retail market. In addition to the higher rates of heat transfer, other advantages are avoidance of periodic shut-downs for defrosting of heat-transfer surfaces, great flexibility in the size and shape of containers, and ease of mechanization to minimize labor cost.

Dehydrofreezing Process Developed Further

The Western Regional Research Laboratory has made further advances in developing its dehydrofreezing process, which includes partial dehydration to reduce weight and volume, followed by freezing and

ordinary frozen-food storage. The potential economic advantage of this process in comparison with ordinary freezing lies in lower transportation and storage costs. Previous reports described effects of variations in processing technique on quality of dehydrofrozen apples and peas. Recent investigations have dealt with (1) comparison of several commercial varieties of apples with regard to suitability for dehydrofreezing, (2) adaptability of dehydrofrozen apples to commercial bakery procedures, and (3) preparation and evaluation of quality of dehydrofrozen apricots and peaches.

In general, it was found that apple varieties considered good for canning or freezing were also satisfactory for dehydrofreezing. Apples used for comparative tests were grade C obtained from the Pacific Northwest except for one lot of California Pippin. Yellow Newtown, Winesap, Jonathan, and California Pippin apples were found well suited to dehydrofreezing; Rome Beauty apples yielded a good product although somewhat soft in texture and low in flavor; both Delicious and Red Delicious apples yielded products considered barely acceptable owing to deficiency in tartness and poor texture.

Results of limited bakery trials in which two large wholesale pie bakeries and one small retail bakery cooperated were most encouraging. Dehydrofrozen apples were found to meet quality requirements for apple pies in every respect. These results are particularly significant since they were obtained by three different bakery procedures. In each instance the baker was impressed with the ease of handling the dehydrofrozen product and with its adaptability to bakery practice. Thus it appears that dehydrofrozen apples will prove commercially acceptable. Contemplated pilot-plant-scale operations should yield valuable information with regard to cost aspects.

Blenheim apricots from one lot of orchard-run fruit were processed into dehydrofrozen, frozen, dried, and canned (solid pie-pack) apricots by standard commercial processing methods except for the dehydrofrozen product. Pies were prepared from these various types of processed apricots, and the baked fruit was evaluated for texture, appearance, color, and flavor. It was found that (1) flavor of the dehydrofrozen apricots was better than that of frozen, canned, or dried apricots, (2) appearance and texture of dehydrofrozen apricots were equal to those of frozen and better than those of either canned or dried apricots, and (3) the color of dehydrofrozen apricots was slightly inferior to that of frozen, about equal to that of canned, and better than that of dried apricots.

Work was started on dehydrofreezing of peaches. While processing procedures developed for apricots appeared generally satisfactory for peaches, difficulty was encountered in removing the product from the dryer trays. As in canning, the clingstone peach is easier to handle and thus appears more adaptable to dehydrofreezing than the freestone. Although generally considered inferior to the freestone peach in flavor, the clingstone yielded a product whose texture, color, and appearance, after being baked in pies, were very attractive.

Submerged Mushroom Culture Yields Tasty Protein Food

Recently it was discovered at the Western Regional Research Laboratory that the spawn of certain strains of edible mushrooms will grow into cobwebby filaments (mycelia) in an aerated, agitated liquid

culture somewhat similar to that used for conventional penicillin fermentation. Culture media prepared from certain fruit and vegetable wastes, from molasses, or from pure sugars and inorganic salts may be used. Contamination with other fungi or bacteria must be prevented.

The mycelium thus produced can be separated and washed in a centrifuge, canned as a cake or slurry, and then sterilized by heating in an autoclave; or it can be frozen. The product resembles bakers' yeast in consistency and appearance, but when cooked it has a typical mushroom flavor. Yields approach those obtained for bakers' yeast (about 50 pounds of dry cells per 100 pounds of sugar), but the fermentation rate is slower.

Nutritional and cultural variables have been studied under an RMA project to determine conditions for optimum growth and flavor, which are necessary for commercial adoption of the process. Satisfactory sources of nitrogen include alfalfa press juice, monosodium glutamate, peptone, a mixture of amino acids, urea, and ammonium hydroxide. With urea as the source of nitrogen, a number of carbohydrates have given good yields. Included are D-glucose, D-galactose, D-mannose, D-fructose, D-xylose, L-arabinose, maltose, sucrose, mannitol, dextrin, and soluble starch. The flavor contributed by the substrate is important and is negligible only with synthetic media. Optimum flavor develops only after full growth, which requires several days. Early harvest yields a product that is bland or almost flavorless.

Two strains of the ordinary mushroom of commerce, *Agaricus campestris*, a white strain and a brown strain, yield mycelium having particularly pleasing but readily distinguishable flavors. These flavors, like most others, are multiple. One strain has a marked nutlike flavor in addition to that of mushrooms. The flavors appear to be fully retained in the canned product. The amounts of thiamine, riboflavin, and nicotinic acid in the mycelium appear to be similar to those found in conventionally grown mushrooms or in bakers' yeast. Mycelium of other strains of mushrooms has also been grown successfully, but without the typical mushroom or other pleasing flavor.

This process has attracted great interest from companies desiring to supplement mushroom-soup stocks and sauces with mycelium for enhancement of the mushroom flavor of their products. Apparently these are the first products to be produced microbiologically with fully acceptable flavor when used substantially undiluted as a foodstuff. The bland products may be used to supply vitamins of the B complex to persons who object to the more pronounced flavor of yeast. The process may be useful in situations in which torula yeast propagation has been used—that is, for the conversion of carbohydrate to protein in tropical countries or under wartime conditions. This process offers the possibility of producing mushroom-flavored mycelium at a substantially lower cost than that of commercially grown mushrooms. Experiments on spray and drum drying of the product are in progress.

Water-Soluble Material in Flour Affects Baking Quality

Differences in the bread-baking quality of flours made from various commercial varieties of wheat are known to every miller. Unfortunately some varieties of wheat best suited for production in certain

sections of the country have poor baking quality, and farmers in these sections may have difficulty in marketing their wheat. Research in the Western Regional Research Laboratory during the past year threw new light on the compositional differences between wheat varieties and justifies the hope that further research will discover not only new ways to use discounted varieties but also better analytical tools for plant breeders who are developing improved varieties.

It was found that in general the bread-baking quality of flour depends in part on the nature and amount of certain water-soluble constituents. This fact may explain why correlation between baking quality and amount and chemical composition of gluten in a flour has not been entirely satisfactory.

Studies were made on flours prepared from a wide selection of wheats and known to range from very poor to very good in baking quality. The samples were obtained through the cooperation of a trade association and the Bureau of Plant Industry, Soils, and Agricultural Engineering. Analyses were made for free and bound lipids (fats), water-soluble nitrogen, and total sulfur in the flours, for bound lipids in the dough, and for total lipids and amino acids in the glutens separated from the flours, but no significant correlations with baking quality were found. The amino acid composition of the glutens was surprisingly uniform.

When the flour samples were fractionated into starch, gluten, and water-soluble material, and these components were then recombined in various ways by methods developed by Karl F. Finney of the Bureau of Plant Industry, Soils, and Agricultural Engineering, it was found that both the gluten and the water-soluble material exert an appreciable effect on baking quality. Omission of the water-soluble material from dough resulted in a poor loaf of bread. Moreover the bread made from a flour of poor baking quality could be improved by substituting water-soluble material from a flour of good baking quality for that originally present. Upon further fractionation of the water-soluble material (by adsorption on an activated clay), it was found that nitrogenous constituents of the fraction, possibly water-soluble proteins, are primarily responsible for this effect.

These observations open up a new and promising line of research to find the much needed correlation between chemical composition and baking quality of wheat flours.

Studies Made of Color Changes During Processing and Storage of Foods

Color is one of the most evident properties of food products and often is an important factor in determining quality. The consumer associates certain colors with freshness and wholesome quality in fruits and vegetables. Thus the extent to which original natural color is preserved during processing and in subsequent storage is one important criterion of success in processing procedures.

The apparent color of a food product depends upon (1) its reflectance or ability to reflect light of various wave lengths (spectrum colors), (2) the quality of the illuminating light, and (3) the sensitivity of the observer's eye. The second and third factors do not relate to the food product and can be standardized. Thus the changes in color of the food samples can be adequately studied by measuring

changes in reflectance characteristics. This is done in an objective way by the use of a photoelectric spectrophotometer or colorimeter. The procedure results in a description of the color in terms of its three sensible attributes: hue, brightness, and saturation.

Various procedures for freezing, dehydrating, dehydrofreezing, and concentrating fruit and vegetable products were evaluated at the Western Regional Research Laboratory by their effects on the original natural colors of the following food commodities.

Peas.—The color of peas stored in the pod changed little during the first 2 days. After this time their color became yellower and lighter and less desirable. The change was more rapid when the peas were stored at room temperature than when iced. The color of frozen and dehydrofrozen peas was as attractive as strictly fresh pod peas. Frozen peas stored at 10° F. for nine months were darker and retained less of their original natural green color than similar peas stored at 0° or −10° F.

Carrots.—Dehydrated carrots treated with starch retained more of their natural orange-red color during storage under various conditions than dehydrated carrots that were untreated or treated with sulfite or ascorbic acid.

Corn.—The color of frozen corn was affected very little by varying the conditions of washing, blanching, and cooling during processing. The color of yellow sweet corn appeared to be closely related to maturity, the more mature corn tending to have a stronger yellow color tinged with orange. It is possible that a photoelectric instrument can be developed for testing maturity of corn on the basis of reflectance.

Rice.—Preliminary studies on the color of rice showed, as expected, the large differences between brown, processed, and white rice which were easily detected by reflectance measurements. Furthermore it was found that rice grains can be easily packed for measurement in a reflectometer, and that the results are sufficiently precise to indicate relatively slight yellowing or darkening that may result from processing.

Lemons.—The degree of ripeness of lemons was particularly striking when measured by a recording spectrophotometer. At about 680 millimicrons the reflectance was 10 percent for a dark-green lemon and gradually increased for light green, silver, and yellow lemons to about 80 percent for the tree-ripened fruit.

Progress Made in Microbiological Studies of Eggs and Egg Products

Continued investigations by the Microbiology Research Division on eggs and egg products were concerned largely with the *Salmonella* (food-poisoning) organisms that have been found in egg powder. Studies recently begun on the effects of various antibiotics on *Salmonella* organisms showed that aureomycin, chloromycetin, and polymyxin B are able to suppress the development of several *Salmonella* strains isolated from egg-powder samples. A wider variety of *Salmonella* types and strains isolated from egg powder will be used in laboratory experiments to learn if the resistance of these strains to antibiotics has been lowered by the heat used in drying the eggs. This work will be supplemented by cooperative experiments with animals by the Microbiological Institute of the National Institutes of Health.

Investigations were also continued under a Research and Marketing Act project and contract, to develop effective microbiological methods for isolating and counting *Salmonella* organisms in eggs and egg products and to determine if *Salmonella* strains isolated from egg powder are injurious to human beings. Examination of between 200 and 300 samples of egg powder by several known procedures indicated the superiority of the modified method developed by the Microbiology Research Division in earlier studies for the isolation of *Salmonella* from egg products. However it was believed that this method could be substantially improved. Toward that end information was accumulated on the role of various substances in meeting the nutritional requirements of *Salmonella.* Among the substances demonstrated to be utilizable as energy sources for growth of *Salmonella* are DL-alpha-alanine, asparagine, sodium citrate, glycerol, lactic acid, and succinic acid. Further studies along this line are expected to provide additional information that will be useful in developing improved methods for the detection of these organisms in eggs and egg products.

Under the contractual phase of the RMA project, which is intended to determine whether *Salmonella* strains isolated from egg powder are harmful to human beings, about 75 human volunteers received complete physical examinations as well as bacteriological check-ups, with respect to blood and stool specimens, prior to receiving test feedings of *Salmonella* cultures. Strains of three *Salmonella* types were tested for their sulfonimide, streptomycin, and chloromycetin sensitivities as well as for ability to infect animals, prior to being used in the feeding experiments. One strain of each of the three types was fed in graded doses to the volunteers in groups of six, and the course of activity was studied. Since this work represents the initial phase in a long-term experiment, far more data must be accumulated before any interpretation can be made of the results.

Although emphasis was placed on studies of the *Salmonella* (food poisoning) organisms in egg powder, the Microbiology Research Division gave attention to other microbiological problems related to eggs and egg products. For example, it investigated the development of a controlled method for the fermentation of egg white in order to improve the uniformity, stability, and general quality of the fermented dried white and thus provide greater outlets for this product. Progress was made in the evaluation of strains of *Streptococci* and *Lactobacilli* that can be used as starter cultures in the pure-culture fermentation of egg white. Experiments with specific *Streptococcus* strains indicated that very rapid fermentation of egg white can be accomplished, as determined by disappearance of carbohydrate and concomitant drop in pH. After the optimum conditions for controlling fermentation have been determined, they will be applied in pilot-plant and commercial-scale experiments.

Turkey Meat Preserved as Frozen Steaks

The need for developing small-weight units of turkey meat for home consumption is apparent when the 18-pound weight of an average dressed turkey is compared with a 4- to 5-pound roast which the average family considers ample at any one time. Increasing the number of forms in which turkey meat can be marketed will help to increase turkey consumption and will provide additional outlets for surplus

turkeys which appear periodically as a result of fluctuations in feed-meat price ratio and the corresponding fluctuations in the level of turkey production. Since the slaughtering of turkeys is a relatively seasonal operation, there is need for frozen storage in order to have turkey meat available throughout the year.

Experiments continued from last year at the Western Regional Research Laboratory have provided a working knowledge of the storage stability to be expected in frozen turkey steaks. In brief, they do not keep as well as frozen whole turkeys, but they keep well enough to permit 6 months' storage under good commercial conditions with practically no loss in acceptability. Crosscut steaks made by sawing the frozen turkeys transversely keep better than the bone-free "cubed" or "knitted" steaks, but both types of steak are good products. These conclusions resulted from experiments in which steaks were stored under varying conditions of packaging and temperature for periods up to 1 year. After periods of 3, 6, and 12 months, the stored steaks were appraised for flavor and tested for peroxides. The development of peroxides was in general well correlated with the degree of off-flavor detectable by the taste panel. As little as 3 months' storage of the steaks at 0° F. or 10° F. in sealed cellophane bags caused definite but not serious deterioration in flavor. At −10° F. definite deterioration did not occur until the steaks had been stored for 6 months. In general, steaks held at 10° F. for 3 months, at 0° F. for 6 months, and at −10° F. for 12 months had equivalent flavor scores.

Aluminum foil, moistureproof cellophane, and polyethylene film were about equally good for packaging of steaks. They provided protection during 6 months' storage, but relatively porous material such as parchment gave less protection. If storage is to last in excess of 6 months, it probably would be better to store the frozen whole turkeys and to prepare the steaks when they are removed from storage, even though preparation of the steaks before freezing might be more efficient as a premarketing detail.

The crosscut steaks were found to be considerably more stable in frozen storage than the bone-free "cube" steaks, apparently because the meat tissue was damaged less in the former. Although not providing uniformity of size from one steak to the next, the crosscut steaks might be favored by some packers because of their longer storage life and because they more efficiently utilize the whole carcass. Others might prefer the bone-free fillet type of cubed or knitted steak. The percentage yield of cooked edible meat on the crosscut steaks, but not their size, was fairly uniform throughout the length of the turkey, ranging from 35 to 53 percent, with an average of 42 percent.

Popular acceptance of this form of turkey-meat product will, of course, depend on educating the consumer as to its convenience and good quality.

Advantages Offered by Precooked Frozen Foods

Precooked frozen foods can provide substantial outlets for large-size turkeys, for malformed but good-quality poultry in general, and for certain vegetables such as potatoes which cannot be frozen to an acceptable product if uncooked. Commercially prepared precooked frozen foods are gaining in favor among consumers. The principal

reasons for this gain are convenience and retention of the quality of freshly prepared foods. Precooked foods not only save work and time in preparation of meals in the home but also reduce waste. In addition to offering convenience to the housewife, the use of precooked frozen foods in restaurants, and especially in hospitals and other institutions where many different food items are needed for therapeutic or other reasons, greatly simplifies the problem of food preparation.

During the past year the Western Regional Research Laboratory began research under an RMA project on the commercial preparation and storage of precooked frozen foods that had not previously received concentrated attention. When three methods were used for cooking turkey, no flavor difference related to cooking method was found in frozen creamed turkey prepared from the meat. A hydrogenated vegetable fat was used in the white sauce. However, when the fat recovered in each cooking method was utilized in preparing creamed turkey, the fat obtained by roasting gave a distinctly rancid flavor to the product. The rancidity was noted immediately after the creamed turkey was frozen and became more pronounced after storage for 6 months at 0° F. The simmering and pressure-cooking methods were more advantageous than roasting, because they could be applied to older turkeys, they consumed less fuel, and they made possible the use of the recovered fat for white sauce without imparting a rancid flavor.

Since precooked frozen foods might be an outlet for surplus poultry of different classes and grades, Leghorn fowls and roosters and colored fowls, roosters, and high-quality roasters were compared for their value in frozen creamed chicken. The only outstanding difference noted was the comparative lack of flavor in the roasters. Again, the results are favorable in that use of the older and less expensive birds is not only permissible but yields a more flavorful product for dishes where adequate tenderness can be effected as in simmering or pressure cooking. Further studies of birds of various grades are planned. A number of storage experiments on precooked frozen turkey, chicken, and vegetables are not yet completed.

Emulsified sauces or gravies which are used in many combination dishes where meats and vegetables are the main ingredients often separate into their components after being frozen and thawed. Usually they will become smooth again if stirred during heating, but stirring damages the texture of the vegetables or other foods that may be frozen in the sauce. Therefore, a sauce is needed that will acquire a smooth appearance with a minimum of stirring during heating. Homogenization of the sauce prior to freezing and the addition of gelatin were found to retard the separation, but these measures do not fully solve the problem. Further studies are planned.

Another difficulty is that many precooked frozen foods used in mixed dishes are too soft after being cooked, frozen, and reheated. Vegetables in particular lack crispness. Experiments with celery, carrots, and peas showed that these vegetables do not become soft and mushy, provided they are not overcooked before freezing and are reheated only until they reach a desirable serving temperature. Excessive heating at either stage, however, has an adverse effect on the texture of all of them. The unusually crisp texture of Chinese water chestnuts, however, is unaffected by either prolonged heating or freezing. The water chestnuts have a very mild flavor that would not detract from

the flavor of any combination dish, and they may therefore be a solution to the search for an ingredient that will retain crispness under various conditions of processing.

Use of Froth-Flotation Pea-Cleaning Process Expanding

Commercial application of the froth-flotation process for cleaning vined green peas, which was developed by the cooperative Fruit and Vegetable Products Laboratory at Pullman, Wash., and was mentioned in the annual reports for 1944 and 1946, resulted in substantial savings to the pea-processing industry of eastern Oregon and Washington in 1948. These savings were produced by reduction of labor required in hand sorting of peas, improvement in the quality of the processed product, and salvage of a considerable tonnage of peas which otherwise would have been discarded because of heavy contamination with nightshade berries. Application of this process to the cleaning of other edible seed crops may add considerably to its ultimate value.

Twenty-three commercial froth-flotation units were in operation in 1948. A food-processing equipment company is now manufacturing froth-flotation units, but most of the units operated in 1948 were constructed and operated by processors who were in close contact with the Pullman laboratory.

Thus far use of the froth-flotation process has been confined largely to processors in the Pacific Northwest who were able to consult with members of the Pullman laboratory staff regarding the construction and operation of the equipment. In order to facilitate wider application of the process a mimeographed circular presenting working instructions for the construction, installation, and operation of the froth-flotation equipment was prepared jointly by the Pullman laboratory and the Western Laboratory. This circular has been widely distributed to interested processors of green peas in other areas.

Avoid Bruising and Delay To Insure Fresh Flavor in Frozen Peas

Research at the Western Regional Research Laboratory has shown that bruising of peas in the viner and undue delay before processing are responsible for off-flavor in the frozen product. Apparently the damaged tissue is subject to not yet understood chemical changes, which, after some time, result in odor and flavor that are foreign to the product. Peas that are shelled carefully by hand do not develop this type of off-flavor, even when processing is delayed many hours, although loss of sugar and other metabolic changes associated with normal respiration do occur. Bacteria do not necessarily contribute to the development of the off-flavor in bruised peas that are not processed promptly. Bacterial counts on samples taken at intervals during the period of holding before processing showed no significant increase in numbers with time of holding. Peas that had been bruised but kept free from bacteria developed the typical flavor at a rate comparable to that observed with peas not so carefully handled.

Deterioration of viner-bruised peas becomes more pronounced with increases in holding time and temperature. Thus, a 1-hour delay at 40° F. between vining and blanching might not result in detectable off-flavor, but a 2-hour delay at 70° F. almost certainly would. These findings prove that viners should shell peas with a minimum of

damage, and that delay and exposure to high temperature between the viner and processing plant be avoided. Obviously, viners should, whenever possible, be adjacent to the processing plant.

The wetting of shelled peas does not appear to aggravate predisposition to off-flavor; accordingly, no exception can be taken to the use of hydrocoolers or icing to effect the necessary lowering of temperature if the product must be transported some distance for processing.

As a result of this work commercial freezers of peas are considering the possibilities of moving viners to the plants. In due course improvement of the quality of the total pack will strengthen the position of frozen peas in the American diet.

Dried Carrots Preserved by Starch Coating

Although the production of dehydrated vegetables has generally slumped since the end of World War II, dehydration of carrots, chiefly for dry soup mixes, is still of some importance.

Before dehydrating or freezing vegetable products it is customary to blanch (scald) them with steam or hot water to inactivate certain enzymes that would otherwise cause rapid deterioration during storage. This treatment alone does not give carrots sufficiently long shelf-life, and it has been the practice to treat subsequently with sulfite in order to achieve greater stabilization. However, even this treatment does not protect dehydrated carrots against loss of color and flavor over a period of months. Packaging the material in contact with nitrogen gas in airtight metal containers is a satisfactory but expensive solution to the problem.

About 2 years ago carrot dehydrators requested the Western Regional Research Laboratory to investigate the value of starch coating on dried carrots as a means of preservation. This technique had been studied by British food technologists, whose work was based in part on observations made by the Western Laboratory on dehydrated carrots in 1943.

In laboratory and pilot-plant-scale experiments dried carrots that had been processed by the usual commercial practice, supplemented by coating with hot 3-percent starch solution directly after blanching, retained color and flavor considerably longer than samples that had only been blanched or had been blanched and sulfited. For example, after storage for 6 months at 85° F. a sample of starch-coated dried carrots still retained its original color and was almost equal in flavor and texture to a sample that had been packed in nitrogen and held at −30° F. for the same length of time. Samples that had not been starch-coated faded and developed undesirable odors and tastes.

Microscopic examination of the starch-coated carrots showed that the starch forms a very thin but continuous gelatinous envelope for each individual piece. Apparently the coating is impermeable enough to inhibit the diffusion of air into the pieces which ordinarily is responsible for the oxidative changes that result in the loss of color and flavor.

Studies with different kinds of starch showed that they are equally effective. For example, only slight differences in stability were detectable in samples preserved with starches from corn and wheat. An unexpected finding was that the carotene in blanched and sulfited carrots was no more stable than that in carrots that had only been blanched

before coating with starch. This is of interest to dehydrators because several States have regulations against the presence of sulfite in foodstuffs.

The technique of starching carrots before dehydration is simple and readily adapted to the processing plant. Thus far, two companies have begun to use the procedure. The result should be less loss through deterioration of product and greater demand for the better product from more satisfied consumers.

TANNIN-BEARING PLANTS

More Learned About Potential Tannin Sources

In cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering work was continued at the Eastern Regional Research Laboratory on the development of additional supplies of domestic vegetable tannins. The materials selected for special study included canaigre, sumac, waste and undeveloped barks and woods, and nut shells. The results obtained in the study of canaigre under an RMA project are given under a separate heading.

Sumac.—The experimental planting of sumac at Beltsville, Md., by the Bureau of Plant Industry, Soils, and Agricultural Engineering under a cooperative program on cultivation of sumac was expanded considerably. Analyses of 121 leaf samples from this planting collected in 1948 showed tannin contents ranging from 28.8 to 41.8 percent for *Rhus copallina* and from 25.9 to 35.5 percent for *Rhus glabra*. The plants have maintained these relatively high tannin contents for several years and so provide a supply of superior planting stock for possible future expanded plantings.

A comprehensive survey of the tannin content of leaves from eight species of domestic sumac from the eastern half of the United States was completed, and the results were prepared for publication as Technical Bulletin No. 986. Average tannin contents and related data for each species are given together with conclusions regarding the effects of sex, sunlight, season, height of plant, and geographic location on tannin content. The bulletin is now available.

Barks and Woods.—Because large quantities of bark containing varying amounts of tannin occur as waste products in the paper-pulp and lumber industries, studies were undertaken of these materials to determine the possibility of their use as commercial sources of tannin. Through the cooperation of the Northeastern Wood Utilization Council and interested industries, samples of these waste barks were provided for study. Much of such waste occurs as mixtures of barks of several kinds which could not be separated economically.

The tannin contents of the barks tested, in percentage on a moisture-free basis, were as follows: Red spruce, 13.0; eastern hemlock, 12.2; fir 3.4–4.1; pine, 6.0–7.3; aspen, 4.9–5.0; maple of various kinds, 1.9–9.4; birch of various kinds, 1.4–5.3; and cherry, 3.2. For bark mixtures the percentages were: Fir and spruce, 2.8–4.9; oak, red maple, birch, and poplar (75 percent oak), 6.9; hemlock and poplar, 6.2; and oak, maple, and birch (80 percent oak), 5.2. The most promising of these barks are red spruce, eastern hemlock, and possibly some maples and pines. Segregation of barks high in tannin would be desirable. Chipping of slabs and limbs followed by mechanical separation of bark from the wood is worthy of study.

The Ozark chinquapin *Castanea ozarkensis* resembles chestnut in some respects. Two samples from a single log showed tannin contents, in percent on a moisture-free basis, to be as follows: Bark, 14.1 and 15.9; wood, 8.0 and 8.4; and combined bark and wood, 8.9 and 9.6. The bark contains a mixture of the hydrolyzable and condensed types of tannin. In the wood the hydrolyzable type of tannin predominates. If this tree is available in sufficient quantity and density of stands to justify commercial utilization as a source of tannin, it deserves more thorough study.

Samples of castanea, quercus, and their hybrids that are under study by the Bureau of Plant Industry, Soils, and Agricultural Engineering for development of blight-resistant trees were examined to determine how their tannin contents compare with that of chestnut wood. They showed percentages of tannin as follows: *Castanea mollissima* (21 samples), 8.8–14.5; *Quercus acutiss* (4 samples), 1.4–1.7; *Quercus dentata* (2 samples), 5.4–5.5; hybrid of *mollissima* and *dentata* (3 samples), 7.3–12.3; and hybrid of *mollissima* and *crenata* (3 samples), 7.1–9.0.

Nut Shells.—Further studies were made on the recovery of tannin from pecan shells. Careful hand separation of hard outer shell from soft liner material had previously shown that the hard outer shell usually contains from 0.4 to 1.2 percent tannin and the liner material from 25 to 48 percent tannin. Mechanical treatments at the Northern Regional Research Laboratory were partly successful in separating ground mixtures of outer shell and liner. Shells from a commercial shelling plant, where some of the liner material had been removed, contained about 5 percent tannin. Mechanical separation gave an outer-shell portion containing 0.3 percent tannin and a mixture of outer shell and liner with 17 percent tannin. Hand separation of this lot of shells gave an outer-shell portion with 1 percent of tannin and a liner portion with 30 percent of tannin. Together with the tannin, some substance is extracted from pecan shells which slows up the combination of pecan-shell tannin with hide substance and forms an objectionable gelatinous sediment in tanning extracts prepared from pecan shells. Further studies are needed to improve the mechanical separation of hard and soft shell parts and to investigate the material causing the gelatinous sediment in tanning extracts.

Production of Tannin From Canaigre Roots Now in Development Stage

The development work on producing tannin from canaigre, *Rumex hymenosepalus*, is being carried on cooperatively as an RMA project by the Eastern Regional Research Laboratory and the Bureau of Plant Industry, Soils, and Agricultural Engineering. The latter is primarily responsible for the agronomic and field production phases, and the former for the laboratory investigations and pilot-scale extraction and processing studies. Experimental and production plantings are located at State College, N. Mex., and Yuma, Ariz., and arrangements are being made for plantings in other places.

Analyses and extraction tests on 49 samples of canaigre gathered by the Bureau of Plant Industry, Soils, and Agricultural Engineering from the southeastern quarter of Arizona and the southwestern quarter of New Mexico confirmed previous tests in showing the superiority of

the red or Arizona-type roots as compared with the yellow or New Mexico roots. The average tannin content of all the red root samples was 39.3 percent with a purity of 68, while for the yellow roots the average tannin content was 28.9 percent and the purity 54. Other points of superiority for the red roots are greater ease of extraction and the production of clearer liquors. A considerable quantity of red roots was harvested for use in experimental studies.

For further study of changes in the roots during growth, samples were collected from two plots at monthly intervals from February to September. Analyses and extraction studies confirmed previous data in showing that roots in the early stages of growth are low in tannin and purity, are difficult to extract efficiently, and give cloudy, unsatisfactory liquors. These adverse qualities disappear when the roots mature.

Drying tests were conducted on shredded canaigre roots in collaboration with the Bureau of Plant Industry, Soils, and Agricultural Engineering at Yuma, Ariz., where sun drying was used and at the Eastern Regional Research Laboratory, where artificially heated mechanical dryers were used.

In sun drying of roots spread outdoors on asphalt or concrete pavement, the results were entirely satisfactory when densities up to 1 pound per square foot were used. When the roots were spread 1.4 pounds per square foot, the results were acceptable but not as satisfactory as when more thinly spread, indicating that 1.4 pounds per square foot is about the maximum amount that can be dried effectively. There was a loss of tannin when the roots were spread on roofing paper. Roots spread indoors gave poor drying results regardless of how thinly they were spread, probably due to the action of molds and other organisms that destroyed tannin and sugars. Results could doubtless be improved by forced circulation of air and occasional stirring of roots. Drying of shredded roots by means of artificial heat and forced circulation of air at temperatures below 150° F. gave a satisfactory product, but drying at temperatures above 150° F. decreased extractability. With the object of lowering drying cost, studies were started on initial drying at temperatures below 150° F., followed by final drying of the nearly dry material at temperatures above 150° F. Preliminary tests to determine the maximum moisture content that may be in roots without molding or bacterial spoilage indicated that moisture in the dried roots need not be less than 15 percent.

Pilot-scale investigations on the extraction of tannin from canaigre cossettes were made by conventional leaching methods in stationary vats with water and with mixtures of water and acetone or isopropyl alcohol as solvents. The results confirmed previous laboratory analyses and showed that when particle size was large enough to permit free percolation of solvent, tannin extraction was incomplete. The mixtures of water and organic solvent gave tannin recoveries of over 80 percent, but an economic evaluation showed that this process would be much more costly than the continuous countercurrent extraction method.

Tests with a commercial continuous extractor indicated that efficient tannin recovery from finely ground roots can be accomplished by continuous countercurrent extraction. By means of this apparatus, 93 percent of the total tannin was extracted with a mixture of isopropyl alcohol and water as solvent. When water alone was used as solvent,

the tannin recovery was somewhat less than 80 percent in the preliminary tests completed thus far.

Fermentation of the sugars without damage to the tannin in liquors extracted from canaigre was successfully accomplished by a specially developed laboratory procedure, and the fermented liquors, clarified by centrifuging and concentrated by vacuum evaporation, were spray dried to yield powdered canaigre extract of satisfactory quality.

For the removal of sugars from canaigre liquors, pilot-scale fermentation tests were also conducted. These indicated that with proper equipment for maintaining aseptic conditions, the *Aerobacter* fermentation is entirely feasible for plant-scale operations. The liquors require only pasteurization at 80° to 100° C., and the fermented liquor can be used to inoculate a fresh lot. Preliminary studies on the recovery of the products of this fermentation are in progress.

Yeasts generally will not grow on canaigre liquor. A few kinds do grow on it and destroy the tannin as well as sugars. Some yeast strains have been found, however, which, with proper handling, can be made to destroy the sugars alone and produce good yields of alcohol.

HIDES AND LEATHER

Microscopic Method for Determining Hide Shrinkage Aids Tanning Studies

When a piece of hide or skin is heated in water it shrinks very much within a small temperature range. The hide constituent principally responsible for the shrinkage is the fibrous protein, collagen. In general the shrinkage temperature is raised by tanning and lowered by swelling agents and by degradation or spoilage of the protein. To determine the effects of tanning agents on hide substance, the Eastern Regional Research Laboratory developed a microscopic method for measuring shrinkage of collagen or leather.

In using the microscopic method, a suspension of the finely divided sample of hide or leather in an ordinary capillary melting-point tube is heated electrically on the stage of a microscope and observed at a magnification of 50 to 75 diameters. The shortening of the collagen fibers that takes place at the shrinkage temperature is so pronounced that even inexperienced observers have no difficulty in recognizing the phenomenon.

Reproducibility of shrinkage-temperature determinations is generally better by the microscopic method than by the conventional method of heating relatively large test strips. This may be associated with rather subtle physical differences within the large specimens—for example, differences in structure or extent of hydration prior to the test. The chance of such lack of uniformity is much reduced in the microscopic specimens. However, when dispersions of thick leather specimens are tested by the microscopic method, shrinkage over a range of temperatures is generally noted, indicating nonuniformity, such as unequal tannage, in the original specimen.

Frequently chrome-tanned and mineral-retanned, vegetable-tanned leathers have shrinkage temperatures above the boiling point of water. For large specimens shrunk in water alone, a test of such leathers requires pressure apparatus which is inevitably bulky and clumsy to operate. By the microscopic method shrinkage temperatures up to

140° C. or higher can be measured conveniently after simply sealing the capillary melting-point tube.

The microscopic method is also advantageous for the rapid evaluation of curing, tanning, or other agents whose effect on collagen or leather may be estimated from the shrinkage temperature. The fine subdivision of the specimen material insures ready accessibility to the reagents and thus a minimum reaction time. Since measurement is made with a microscope, it is of course implied that only small quantities of materials are needed.

Further Progress Made in Alum Retannage of Insole Leathers

Shoe insoles are usually made of vegetable-tanned leather, because it has physical properties most suited for the purpose. The insole forms the foundation upon which the shoe is built. Because large percentages of the failures in military shoes were caused by failures of insole leather, it is highly important that a process for the production of more durable insole leathers be developed. Alum-retanned, vegetable-tanned insole leathers have been suggested, and wear tests conducted by the Quartermaster's Office have shown these leathers to be more serviceable than unmodified vegetable-tanned leathers.

Previous studies at the Eastern Regional Research Laboratory showed that vegetable-tanned insole leather can be successfully retanned with alum to yield an area-stable leather containing over 3 percent of aluminum oxide and having a shrinkage temperature above 100° C. The retanning process developed in the laboratory required from 3 to 5 days, depending upon the thickness of the leather, and when adapted to tannery use would necessitate additional equipment and space. To perfect this process, adjust it to tannery conditions, and facilitate its adoption for use in commercial tanning, further studies were conducted.

A "dry-dipping" process, considered for its time-reducing possibilities, was only partially successful in producing an area-stable leather and would require the use of considerable tannery-floor space that might not be available.

Commercial vegetable-tanned belly leathers from three tanneries were alum-retanned by the laboratory process and gave leathers which had shrinkage temperatures ranging from 123° to 133° C. and showed no reduction in area when boiled in water for 3 minutes. However these leathers appeared to be somewhat softer and spongier than commercially produced vegetable-tanned insole leathers. To overcome this condition, a lighter alum retannage was applied to six vegetable-tanned, unfinished belly leathers obtained from a commercial tannery in the wet condition. After being retanned, these leathers were returned to the tannery for bleaching, oiling, and finishing. The finished leathers contained 2.4 percent of aluminum oxide and had a shrinkage temperature of 108° C. They were area-stable to boiling water except for slight shrinkage of the grain layer. The leathers were subsequently cut into insoles at a sole-cutting plant and were reported to be of satisfactory solidity and suitable for insoles.

Considerable interest in the commercial application of the alum-retanning process has developed in the tanning industry. At the request of a heavy-leather tanner, a preliminary full-scale alum-retannage test was applied to 200 vegetable-tanned belly leathers. Although available equipment and tannery conditions left much to

be desired for the test, the leather produced was acceptably retanned. The aluminum oxide content ranged from 1.6 to 2.7 percent and shrinkage temperatures from 102° to 106° C. The area-stability of the leathers was generally good, the thin leathers showing no shrinkage in boiling water and the thicker leathers only a slight shrinkage of the grain layer.

Considerable progress was made in adapting the retannage process to tannery conditions. Recent laboratory tests resulted in an improved procedure by which the alum-retanning liquors are applied to the leather after being bleached and while the oiling and filling operations are in progress in the oiling drum. The process can thus be fitted into regular tannery practice without any material change in equipment and only a moderate increase in the regular operating time of the oiling drums. By utilizing the 5-day period normally required for drying leather for the fixation of alum as well as for drying, time is conserved without interference with regular tannery schedules.

Laboratory tests with 25 bellies and tannery tests with 200 bellies per drum load are being conducted to perfect the details of this procedure.

ANIMAL FATS, DERIVATIVES, AND PRODUCTS

New Constants Found for Physical Analysis of Fats

Last year's report mentioned a new procedure that was developed at the Eastern Regional Laboratory for fractionating and isolating pure natural unsaturated acids of fats. It was based on the principle of chromatography. During the past year, pure unsaturated fatty acids, isolated in their natural form for the first time by means of this new procedure, served as standards for establishing precise constants for use in the spectrophotometric method of analyzing fats and oils. Following presentation of a paper before the American Oil Chemists' Society in November 1948 on the subject of revised constants and calculations for the spectrophotometric method, the Committee on Spectroscopy is giving serious consideration to the adoption of these new constants for this important and widely used physical method of fat analysis.

Technical Oleic Acid Improved

Small pilot-plant investigations were started at the Eastern Regional Laboratory on a process believed to be suitable for the economical production of an improved technical grade of oleic acid from inedible animal fats. Statements regarding the availability of samples of this new product for investigation and practical evaluation in industry were published in several chemical journals. A large number of inquiries were received in response to these notices, and information about the process and working samples of the product were supplied to all persons requesting them. It seems certain that this improved oleic acid will find important new uses and expand the utilization of inedible animal fats.

New Polymers Made from Fatty Acids

In continued research at the Eastern Regional Research Laboratory on the preparation of polymerizable esters from long-chain fatty acids, the vinyl, 2-chloroallyl, allyl, methallyl, and similar unsaturated-

alcohol esters of caproic, caprylic, pelargonic, capric, lauric, myristic, palmitic, stearic, and 10-hendecenoic acids were prepared and characterized.

The polymerization and copolymerization of some of the vinyl esters were studied in detail. These esters are readily polymerized, and, with the exception of polymerized vinyl hendecenoate, the polymers are themoplastic materials that dissolve in organic solvents and vary in consistency from viscous liquids or soft, rubberlike masses to solids having low melting points. Vinyl hendecenoate, however, readily undergoes cross-linking reactions to yield insoluble polymers and copolymers. Copolymers of vinyl acetate with the vinyl esters of the saturated fatty acids are soluble in organic solvents and vary from hard, glasslike materials to rubberlike masses, as the content of long-chain vinyl ester is increased.

Molecular weights of the polyvinyl esters of long-chain fatty acids, in the absence of chain-transfer agents, are usually in the range of 200,000 to 400,000, although molecular weights of over 1,000,000 have also been obtained. By employing suitable modifiers, the degree of polymerization can be controlled to yield low polymers, even dimers. The low- or intermediate-molecular weight products are potentially useful as plasticizers for resins and additives for lubricants.

WOOL AND WOOL GREASE

Basic Knowledge of Wool Fibers Extended

The first full year of research on wool at the Western Regional Research Laboratory comprised a comprehensive investigation of the fundamental physics and chemistry of wool. The project, financed by Research and Marketing Act funds, is designed to discover the basic facts upon which technological improvements in wool manufacture can be based, and thus to assist in the restoration of American wools to a fully competitive position in textile markets.

More than 60 chemically modified wools were prepared, and their physical properties were examined by newly developed techniques. Several showed significant alteration of properties—changes that may prove to be either beneficial or detrimental after more thorough evaluation. For example, treatment of wool fiber with beta-propiolactone under certain conditions imparted greater stability to the disulfide bonds that account for most of the strength and resilience of wool fibers. The treatment also changed the type of stress-strain relationship in an unusual manner and imparted the ability to form felts having increased strength. This behavior was surprising, because the surface character of the fibers—that is, the scaliness—appeared to be unchanged. The crimp and kinkiness of the fibers were, however, diminished. Further study of this chemical treatment and others can be expected to disclose new and valuable textile effects.

During the growth of a wool fiber, long, slender protein molecules are laid down side by side and are linked with their neighbors at intervals to form the strong, elastic fibrils constituting the mechanical units of which the fiber is built. The primary molecular chains are, in general, much stronger than the cross links that bind the chains together. The nature and properties of these cross links, therefore, have been subjects of special study. Disulfide bonds, salt bonds, and hydro-

gen bonds have been broken gradually by controlled chemical reactions on fibers under stress, and the gradual losses of strength and elasticity have been measured. The resulting information provides a basis for comparing different types of wool and may point the way to methods for improving wool quality.

The orderliness of arrangement of molecules in the wool fiber has been measured by means of X-ray diffraction, and further information about molecular structure has been obtained by determining dielectric constant at microwave frequencies and low temperature. The latter measurement appears to offer a new means for nondestructively distinguishing wool from other kinds of fiber.

A simplified model of the wool fiber has been prepared by polymerizing cystine, one of the important amino acids of wool. A substance of this kind, free from many of the structural complexities of wool fibers, can be used to test the validity of theories connecting strength and elasticity with molecular structure.

Under the terms of an RMA research contract the Textile Research Institute of Princeton, N. J., has undertaken to investigate the relaxation of wool fibers at different stages of processing to get fundamental information needed to develop means of producing fine woolen fabrics from medium grades of wool.

Wool Grease Separated Into Components

Research on the composition, properties, and uses of wool grease was begun recently at the Eastern Regional Research Laboratory under an RMA project. A procedure suitable for laboratory use was developed for saponification of wool grease. Substantially complete saponification was accomplished in 12 hours by agitating a solution of wool grease in isopropyl alcohol with excess of barium hydroxide solution at reflux temperature. Within 5 minutes after the base was added, roughly 55 percent of the original esters were saponified, but only about 4.5 percent of the combined cholesterol known to be present showed up as free cholesterol at that time. This indicates that the cholesterol esters are more difficult to saponify than the other esters, which fact may be useful in developing procedures for isolating the various types of combined alcohols.

Molecular distillation was carried out successfully on a sample of neutral wool grease. About 62 percent of the material distilled between 250° and 275° C. at an absolute pressure of 2 to 4 microns. The fraction distilling between 250° and 255° was mostly liquid, while the other fractions were harder than the original grease. These results indicate that some separation of the constituent esters of wool grease can be accomplished by high-vacuum distillation.

PROTEINS AND PROTEIN PRODUCTS

Physically Modified Proteins Remain Porous to Water Vapor

The basic investigation at the Eastern Regional Research Laboratory on the chemical groups responsible for the uptake of water vapor by proteins (hygroscopicity) has given new information about the structure of proteins. Since such a structure was proven for cellulose and many other high polymers it had previously been assumed that the molecules of proteins are so tightly packed that molecules

of water cannot penetrate between the chains. Experiments carried out on wool, silk, and ovalbumin, representatives of the major types of proteins, showed that the sorptive centers are accessible to water vapor. Wool and silk, for example, can be dissolved in strongly polar solvents and reprecipitated as powder without affecting this property. Ovalbumin, water-soluble in its original form, can be converted to the denatured form, insoluble in water, and yet leave its affinity for water vapor essentially the same. These results are not only of theoretical interest in studying proteins; they also show that physical treatments involved in the production of fibers or plastics from proteins cannot be expected to modify the uptake of water vapor. This ability to combine with water vapor is a desirable property in textile applications, for it produces a warm and dry feel in clothing. However, in plastics it is undesirable, for it leads to alternate swelling and shrinking and to consequent checking and cracking of the plastic article.

Casein Textile Fiber Stabilized to Boiling Dyebaths

Continued improvement in the physical and chemical properties of the textile fiber made from casein has been made at the Eastern Regional Research Laboratory. Heretofore artificial fibers made from casein or other protein have been used in the form of staple (short lengths) for blending with other fibers in spinning yarns. Recently the Eastern Laboratory made continuous-filament casein yarn for the first time and found that it has potential industrial value because of unique properties. Its stability is markedly improved by a newly developed treatment. This treatment, unlike the acetylation treatment formerly employed, does not require the use of inflammable organic solvent with attendant higher cost and possibility of danger to operators. It comprises steeping of the fiber in a 10-percent solution of formaldehyde containing an acid, removal of excess solution by centrifuging, drying at gradually increasing temperature, and finally baking at 110° C. The finished fiber possesses truly rubberlike elasticity, which results from its cross-linked structure, and is warm and smooth to the touch. These properties bring nearer the possibility of developing an artificial all-protein yarn for use in knit goods. Details of this process have been made public; commercial application is being seriously considered by at least one company.

Uses for Casein Bristle Expanded

Important new commercial uses have been developed for casein bristle. Haircloth, used for stiffening the lapels of men's coats and for similar purposes, has been successfully made with casein bristles in place of horsehair. The machinery and the process used in making haircloth with horsehair were used with slight modification. The stiffness of haircloth made with casein bristles could be varied widely by using lots of bristles having different diameters. The flexibility of this stiff casein cloth is good, as shown by a lack of breakage during mechanical flexing. Approximately 300,000 pounds of horsehair are used annually in this country in making stiff cloth. Since processed

horsehair for making stiff cloth is largely imported at several dollars a pound, the use of casein bristles in making this material offers a favorable economic advantage as well as the possibility of various degrees of stiffness.

Another new outlet for casein bristle is its use in automobile air filters. A manufacturer whose factory was originally built to manufacture casein bristle for paint brushes by the process developed by the Eastern Regional Laboratory, is supplying curled casein bristle to a manufacturer of air filters. The curled filament is mixed with wool, and the mixture, after being impregnated with tricresyl phosphate, is placed in the filter cartridge. The entire assembly is designed for periodic replacement. It is estimated that this one use will supply an outlet for at least a million pounds of casein annually.

The same manufacturer has produced coiled casein bristles which have pronounced resiliency, suggesting that such material could be used in furniture stuffing and other padding. Tests have indicated that the resiliency of casein bristles is durable, and samples of this material are being further tested for utility in various articles of furniture by upholsterers.

PINE GUM, DERIVATIVES, AND PRODUCTS

Ninety Percent of Nation's Pine Gum Cleaned by Bureau's Process

Twenty-seven central gum-naval stores plants, producing 90 percent of the country's gum turpentine and rosin, now use the Bureau's improved process for cleaning pine gum. This process in commonly known and referred to by the industry as the "Olustee process," because it was developed at the Naval Stores Station in Olustee, Fla. Four plants, in addition to those previously reported, adopted the process during the past year, leaving only one that uses another method of gum cleaning.

Such wide use of the process, which gives a higher yield and better grades of rosin than fire-still distillation or any method of cleaning previously used, has added considerably to the incomes of processors and, consequently, to the income of the 40,000 gum farmers of the Southeast. Since the processors now are in active competition with each other in buying the crude gum, because they can depend on obtaining more valuable products from it, they are paying the farmers higher prices. Moreover the improved quality obtained by use of the "Olustee process" results in higher values of rosin that is placed with the Commodity Credit Corporation for loans. Estimating, conservatively, that the rosin is about two grades better than could be obtained directly by fire stilling or by other cleaning methods, the value of the rosin used to get loans in 1948 was increased by about $91,000.

Naval Stores Station Saves Money for Pine-Gum Processors

Increasingly during recent years the gum-turpentine industry has looked to the Naval Stores Research Division for technological, chemical, and engineering assistance. The Naval Stores Station, located in the heart of the gum processing industry, received nearly 20 percent more calls for help in the 1948–49 season than during the preceding year. Its staff gave information to more than 450 visitors,

held 31 conferences with processors at the Station, and made 77 visits to plants. In addition, much information was supplied by correspondence.

Not only was the Station called upon to assist in the installation and initial operation of new equipment when plants converted to the modern "Olustee" production methods, but its efforts also resulted in substantially improving the efficiency of plant operations in a number of instances. For example, advice on proper boiler operation and on the minimum use of steam to produce high-quality products by steam distillation enabled one large processor to reduce his fuel-oil consumption per barrel of gum processed from 7 to 4 gallons. A saving in operating cost was effected at another plant by introducing improved techniques of filtering crude gum to obtain maximum efficiency, thereby reducing the time-consuming and expensive operation of cleaning the press and changing the filter medium. Another processor was assisted in saving an entire lot of turpentine which had been contaminated with foreign soluble matter by supplying advice on a procedure for rerunning it. An entrainment trap for resin acids was designed which enabled processors to reduce acidity of turpentine to well within the requirement for Government loans.

The dollars-and-cents savings resulting to industry from technological and engineering assistance of this kind has been substantial during the past year. In addition, much fundamental information was provided to assist professional engineers, as well as processors, in problems of plant and equipment design and in planning for more efficient operational procedures.

Turpentine Quality Improved by Reducing Acidity

Within the past year more attention than ever before was given by turpentine processing and packaging plants to the reduction of acidity as a means for improving the quality of turpentine. This resulted in part from complaints by large dealers and consumers about the poor storage quality of some turpentines. In addition, the high acidity of turpentine caused the Commodity Credit Corporation to establish a maximum limit for acidity of turpentine accepted for Government loans in 1948.

In order to give the industry immediate assistance in its efforts to reduce turpentine acidity, the Naval Stores Research Division developed two improved processing procedures. One of these, now in use at several plants, separates the low-acid middle cut from the distillation, that is, the best turpentine, for shipment and returns the poorer quality fore-run and tailings to the still for reclaiming. The other procedure uses a column-type separator in the turpentine-vapor line between the still and condenser to remove the entrained resin acids. In pilot-plant tests a separator of this kind reduced the acid number of turpentine from 1.0 to 0.1, which is very good for commercial turpentine. Poor turpentine may have an acid number as high as 3.0. A quick method for determining turpentine acidity by means of a chemical test was also devised. This control method saves considerable time in checking acidity for plant-control purposes and can be used by any plant operator, even one without technical training.

New Industrial Outlets Sought for Turpentine

The industrial consumption of turpentine of all kinds was about 26 percent less last year than the year before, and American stocks of turpentine increased correspondingly. A marked drop in the use of turpentine for chemicals and pharmaceuticals, which accounted for most of the decline in total consumption, is directly traceable to greater availability of competitive synthetic chemicals supplied by the chemical industry.

These facts clearly indicate that new chemicals from turpentine are needed if the competition of synthetic chemicals obtained from other sources is to be met with any degree of success. Research to meet this need was intensified during the past year.

Tests were conducted, in collaboration with the Office of Rubber Reserve's research laboratory at the University of Akron, on the use of various terpene peroxides as catalysts in the production of "cold rubber," that is, synthetic rubber produced at lower temperatures than those commonly used in the past. Suitable methods for the production of such peroxides from various terpenes obtainable from turpentine were investigated, and some of these peroxides were found to be efficient catalysts.

The peroxide used most extensively at the present time is obtained from petroleum. Development of suitable peroxides from turpentine would not only supply a substantial market for turpentine components but would also supply a renewable source of these highly strategic materials and help relieve demands upon the petroleum industry in case of a national emergency.

Other work looking toward chemical utilization of turpentine is in progress.

More Fundamental Knowledge Gained on Turpentine

Much fundamental knowledge on the composition and properties of gum turpentine is needed for the development of a chemical industry based on that material. Additional knowledge of this kind was obtained by the Naval Stores Research Division during the past year.

Studies were made on the composition of turpentine obtained by various methods from several species of pine and on the effect of different methods of getting gum from the trees (by regular chipping or by chipping supplemented with acid or fungus stimulation). The composition of turpentine samples obtained from various American pines differed markedly. The components of turpentine obtained from southern pines are chiefly alpha-pinene and beta-pinene, but the proportion varies greatly. Thus turpentine from the slash and longleaf pines commonly used for turpentining operations contains about 60 percent of alpha-pinene and 30 percent of beta-pinene; turpentine from Virginia pine contains nearly equal proportions of each; turpentine from loblolly pine contains only 10 percent beta-pinene and 70 percent alpha-pinene; and turpentine from sand pine contains about 20 percent alpha-pinene and 70 percent beta-pinene. In comparison, turpentine from the Ponderosa pine of the West contains only about 20 percent of alpha-pinene and beta-pinene combined. Such information on the composition of the turpentine obtainable from various species of pine is obviously essential for the proper chemical utilization of turpentine.

Fundamental research to elucidate the chemical structure or molecular architecture of compounds provided some entirely new information on various derivatives obtainable from turpentine. During the past year the structure of the addition compound of beta-pinene and carbon tetrachloride, a new product referred to in last year's report, was conclusively determined. Knowledge of the molecular structure of compounds of this type is needed to guide their utilization and for the development of additional chemical products from turpentine.

Good Rosin Obtained From Loblolly, Sand, and Virginia Pines

The investigation of several species of pine trees other than the southern slash and longleaf pines as sources of pine gum for rosin and turpentine products, which was begun last year in cooperation with the Forest Service, was extended, and some extremely valuable information, not heretofore available, was obtained.

In addition to rosin from the gum of Virginia pine trees, which was examined in 1948, samples of rosin obtained from loblolly pine and sand pine were analyzed. Rosins prepared from the gum of all three species of pines appeared to be entirely satisfactory for commercial utilization, differing only slightly from the commercial rosins now available.

As previously found for slash and longleaf pines, fungus-stimulation of gum flow apparently caused no damage to the rosin of these three varieties. When samples from fungus-stimulated trees were compared with samples from trees on which such stimulation was not used, no appreciable difference could be detected. Thus it appears that fungus-stimulation might be a means of increasing the flow of gum from Virginia, loblolly, and sand pines to give them commercial importance as sources of rosin and turpentine. In the past these varieties were not considered worth investigating because of low yields of gum.

RUBBER AND RUBBERLIKE PRODUCTS

Progress Made in Extraction of Rubber from Guayule Shrub

Research is being conducted by this Bureau at the Rubber Research Field Station in Salinas, Calif., as a part of a broad program of preparedness under the Strategic and Critical Materials Stock Piling Act of July 23, 1946, to meet emergency needs for natural rubber in the event of war. The major objective of these investigations is to develop new or improved processes for the extraction of rubber from domestic rubber-bearing plants, to translate such processes into factory-scale operation, and to develop means for utilizing byproducts of rubber extraction in order to increase the economic feasibility of domestic rubber production.

This work is coordinated with the research program of the Bureau of Plant Industry, Soils, and Agricultural Engineering on domestic rubber bearing plant improvement. Thus far the investigations on rubber extraction have been wholly confined to the guayule shrub, since this plant appears, at least at this stage of development, to be the most promising from the standpoint of yield and cost of production.

Milling lush guayule shrub

In the past, when mature guayule shrub was ground in pebble mills for extraction of crude rubber in the form of "worms," it was standard factory practice to "condition" the shrub before it was milled. Conditioning consisted of various degrees of field curing, followed by baling and storage in warehouses for indefinite periods. Completion of conditioning was judged wholly on moisture content, and it was believed that optimum moisture content for processing was 13 to 15 percent. In the later stages of conditioning, it was customary to pass the chopped-up shrub through a rotary drier in a blast of hot air to bring about a final reduction in moisture. This drying caused the moisture in the finer shrub particles to fall far below the desired percentage, while the larger pieces were wetter than desired. Such conditioning was admittedly imperfect, but was regarded as essential to maximum recovery of rubber.

The rubber in fresh guayule shrub is present as latex, and it has been demonstrated that such latex will coagulate very rapidly upon rupture of the cells and exposure to air, regardless of the amount of moisture in the shrub. Recent work has demonstrated that freshly harvested shrub with a moisture content as high as 40 percent can be pebble milled readily in batches with yields of rubber in excess of 95 percent of the total amount in the plant. Exposure to air with resultant coagulation of the latex is accomplished by first crushing thoroughly with rolls that break down the soft parenchyma tissue in which the latex is found. This treatment is followed by hammer milling of the crushed flakes to provide additional opportunity for aeration of the latex. A period of only a few hours after hammer milling is adequate to prepare the material for pebble milling.

Although a process for guayule-rubber extraction based on milling of lush (freshly harvested) shrub has not been completely developed as yet, a number of significant advantages of this practice are apparent by comparison with the conventional procedure.

First, harvesting and storage costs would be lowered. When the shrub is to be milled immediately, harvesting is visualized as being a "once-over" job, whereas under the old procedure the shrub had to be dug, windrowed with a sidecasting rake, and left for several days of field curing. Later it was baled. Since the material allowed to dry in the field becomes tough and unmanageable in the baling presses, the cost of maintaining bailing equipment is higher. Lush shrub bales more easily; moreover the bales do not have to be put in storage warehouses which are normally required for completion of shrub conditioning under the old procedure.

Second, lush shrub could be processed more easily than dried shrub. Defoliation is more readily accomplished; the material is more easily freed from dirt; and the softer tissues of lush shrub can be handled more easily in cutters, crushers, and hammer mills with less maintenance cost than can dried materials.

Third, a rubber of higher quality would be obtained. The crude rubber from pebble milling of lush shrub contains only about half as much insoluble matter as does the rubber from dried and stored shrub. This is important, because one of the principal objections to the uses of guayule rubber in the past has been its high content of insoluble matter, which consists primarily of fragments of woody

tissue that weaken the rubber. It is also probable that rubber recovered from lush shrub is more uniform, since it does not have a chance to become degraded during the conditioning process.

Fourth, over-all yield of rubber should be increased. In a commercial extraction plant, as operated by the Government under the emergency rubber project, rubber recovery ranged from 64 to 80 percent and averaged about 70 percent during typical periods of operation. These recovery figures were based on the rubber content in the shrub at the time of milling and not on the rubber that was present when the shrub was harvested. This distinction is a critical one, since it is known that under certain conditions of field exposure the shrub may lose as much as 50 percent of its original rubber content. Comparatively low yields by conventional milling, together with variable losses during conditioning or storage of shrub, present an unfavorable picture of the old procedure. Lush milling of shrub thus appears very promising as a major improvement over the conventional method of extracting guayule rubber.

Deresination of shrub

Another development of promise, particularly with respect to improvement in the quality of guayule rubber, is deresination of the shrub prior to milling. Typical crude guayule rubber, as produced commercially and under the emergency rubber project, contains about 70 percent of rubber hydrocarbon, 20 percent resins, and 10 percent insolubles. Such rubber, because of its high content of resins and insoluble matter, is regarded as inferior and lacks uniformity in quality. The resins also appear to be at least partly responsible for the poor aging characteristics of guayule rubber. Therefore the removal of resins from the shrub prior to milling or from the rubber after milling would offer distinct possibilities of greatly improving quality, uniformity, and aging qualities.

Deresination of shrub by acetone extraction resulted in the recovery of crude rubber with a resin content as low as 0.5 to 1.0 percent. Pure-gum vulcanizates of deresinated rubber had tensile strengths of 4,000 pounds per square inch or better, as compared to about 2,500 for the corresponding vulcanizates of ordinary resinous rubber. Other physical properties remain to be determined before the quality of deresinated guayule rubber can be adequately described. It appears, however, that deresinated guayule rubber, when properly handled and processed, approaches Hevea (plantation) rubber in quality.

Lactoprene EV Rubber Manufactured Commercially

In last year's report it was stated that the acrylic rubber developed by the Eastern Regional Research Laboratory and named Lactoprene EV because it can be derived from lactose (milk sugar) had created great interest in industry because of its superior heat resistance and potential usefulness for special applications. The outstanding properties of Lactoprene EV include long flex life, low permeability to gases, and excellent resistance to deterioration by dry heat, oils, sunlight, ozone, and oxidation.

Because Lactoprene EV was so promising as a specialty rubber, a rubber manufacturer began making it on a pilot-plant scale in March 1948. This firm also evaluated the product in comparison with other

synthetic rubbers. In September 1948 it announced the commercial production of the new rubber, first under the name Polyacrylic Ester EV and later as Hycar PA–21. It also published a booklet describing vulcanization recipes, processing methods, special properties, and suitable applications for the new product. According to reports from the trade the new rubber is being received favorably and its commercialization is proceeding satisfactorily.

THERAPEUTIC AND OTHER BIOLOGICALLY ACTIVE SUBSTANCES

Rutin Now Established as Important Drug

Rutin has received widespread recognition as an important addition to the therapeutic agents available for the treatment of diseases. Its use by physicians has expanded until it is now obtainable everywhere in this country and in many foreign lands. One evidence of its acceptance by the medical profession is that it has been accepted for inclusion in the next edition of the National Formulary, an official list of drugs and formulas which, with the United States Pharmacopoeia, sets up the legal definitions for recognized drugs. In preparing the definition of rutin, members of the staff of the Eastern Regional Research Laboratory played a prominent part.

Additional evidence of the growing importance of rutin is indicated by the increasing number of publications concerning rutin that appear in medical and scientific journals and report its applications for diverse conditions related to hemorrhagic diseases.

Rutin manufacturers report a slow but steady increase in the demand for this drug. There is active competition in this field, with the gratifying result that the price of pure rutin has been lowered several times during the past year with reflected reductions in the retail price. The high price of rutin in the past has been an obstacle to expanding use of the drug. Rutin is now being prepared by pharmaceutical companies in England, Denmark, Sweden, Switzerland, Australia, China, and possibly other foreign countries.

The Eastern Laboratory developed a modification of the process for extracting rutin from green or dried buckwheat plants by means of which the operating time is much reduced and a better quality of rutin is produced in the first stage than was hitherto possible. The modified process, which should result in considerable economy in manufacture, has been adopted by practically all drug manufacturers operating in this field.

Cooperative experimental work on the variety of buckwheat and the cultural practices that produce the greatest yield of rutin, conducted by the Pennsylvania Agricultural Experiment Station and the Bureau of Plant Industry, Soils, and Agricultural Engineering, was completed during the year, and the results are being tabulated for publication. The work showed very clearly that Tartary buckwheat is superior to Japanese buckwheat, the common variety grown for buckwheat flour, in that it develops a higher content of rutin and retains it over a longer period. Tartary buckwheat may be harvested any time after 45 days of growth with no loss in the per acre yield of rutin, a fact that is important when inclement weather delays harvesting.

Rotary Alfalfa Dryer Used To Prepare Buckwheat Plants for Year-Round Rutin Extraction

The drying of Tartary buckwheat plants and the production of buckwheat leaf meal, to be sold to drug manufacturers for the year-round extraction of rutin, can be accomplished in a rotary, direct-fired alfalfa dryer with an inlet temperature around 1,200° F., according to experiments with a standard farm-size dryer at the pilot plant of the Eastern Regional Research Laboratory. This type of dryer is both cheaper in first cost and more economical to operate than the low-temperature, conveyor-belt type; so a slightly higher loss of rutin could be tolerated. The experimental results indicated that a farmer or commercial alfalfa-meal producer who owns a rotary-dryer outfit for alfalfa could use it to produce good buckwheat leaf meal, perhaps after installing a simple vibrating screen.

When harvesting buckwheat on sunny days it proved advantageous to let the mowed but unraked plants lie in the field until a few of the leaves commenced to get brittle. The plants were then picked up by a hay loader and chopped after their arrival at the dryer. If drying was by the "fractional" procedure described below, the preliminary "wilting" reduced fuel consumption at the dryer by 40 percent and made possible the production of nearly 60 percent more leaf meal per hour. The total loss of rutin, from freshly harvested plant to dried meal, was not increased. However, considerable loss of rutin resulted on cloudy or very humid days when the drying rate was slow. Usually 3 hours' exposure in the field was sufficient. It is believed, though not experimentally proven, that wilted plants should be dried as soon as possible. Thus either unfavorable weather or night operation of the dryer would necessitate drying the plant without wilting.

It was shown that the "fractional drying" procedure already recommended for the belt dryer is also highly desirable for the direct-fired rotary dryer. In this procedure the drying is carried only far enough to embrittle the leaf and flower tissues while the stems remain moist and tough. A centrifugal fan draws the gases and the fractionally dried plants from the dryer and sends them to a cyclone separator from which the gases escape at the top and the plant material falls at the bottom. A second fan picks up the plant material with a stream of cold air and sends it to a second cyclone separator. In passing through the fans and separators the brittle leaf and flower tissues are separated from the stems and broken into small fragments. The mixture is conveyed to a vibrating screen to remove the stems, which are discarded because they contain practically no rutin. The leaf meal passes through the screen into a hopper and is bagged for sale.

The advantages of fractional drying over the total drying that is customarily used for alfalfa are considerable. The quantity of rutin obtained per ton of plant harvested is considerably greater, and the quantity of material that a given dryer can handle per hour is 40 percent greater for wilted plants or 30 percent greater for fresh plants. The fuel consumption per ton of material is one-third less. Finally, since buckwheat leaf meal is customarily priced in direct proportion to its rutin content, the removal of the useless stems, which usually constitute one-third of the total weight, increases the price and reduces bagging and shipping costs.

When fractional drying with an inlet temperature of 1,200° F. was used, about 65 percent of the rutin in the fresh buckwheat plant was retained in the meal, whether or not the plants were previously wilted. With wilted plants the fuel-oil consumption was only 120 gallons per ton of leaf meal produced, or 12 gallons per ton of fresh plants harvested. With fresh plants the corresponding figures were 200 and 20. When an inlet temperature of 900° F. was used, the rutin recovery was the same, but the drying capacity was reduced by 40 percent or more, and the oil consumption per ton increased by 20 percent for fresh plants.

Three Forms of Beta-Carotene Determined by New Method

The beta-carotene in vegetable foods and green feeds is important nutritionally, because it is a precursor of vitamin A. However it exists in several molecular configurations, called stereoisomers, which differ markedly in their vitamin A activity. Most of the beta-carotene of plants consists of three stereoisomers—all-trans, neo B, and neo U—the vitamin A activities of which are in the ratio of 100 : 53 : 38 when fed to rats. Recent work with chicks showed that the vitamin A activities of the all-trans and neo B forms resemble closely their respective activities in rats.

Heretofore only the total beta-carotene content of plant materials could be measured by existing analytical methods. An attempt by the Association of Official Agricultural Chemists to correlate results of one tentative method for the separate determination of the principal isomers gave such divergent values that the work was discontinued, pending further developments. At the Western Regional Research Laboratory, in connection with research on alfalfa, a chromatographic procedure was developed for measuring the relative amounts of the three most abundant stereoisomers of beta-carotene. This method makes it possible for a single operator to determine the relative amounts of the stereoisomers of several samples in a few hours.

The artificial dehydration of alfalfa and other forage plants for feed results in desirable qualities, such as greener color and higher protein content. The primary objective, however, is to reduce the loss of carotene, which is 60 to 85 percent of the original quantity in sun drying and only 10 to 15 percent in dehydration.

Since dehydrated alfalfa meal contains relatively large amounts of carotene as compared to other mixed-feed components, it is used by the feed industry primarily for its vitamin A value. Biological assay of this constituent is impractical, not only as regards cost, but also because of the long time required. Present chemical methods for measuring total carotene express the results in terms of the parent or all-trans form. Such analyses may indicate a carotene content as much as 30 percent higher than that corresponding to the actual nutritional value of the carotene present. In fresh alfalfa 85 to 90 percent of the total carotene has been found to be in the most active form. However in many dehydrated alfalfa meals this figure has been reduced to about 40 percent, which means that the product has a substantially lower nutritional value.

The new analytical method will aid in learning what conditions of processing result in retention of carotene in its more active form. Preliminary results have shown that during cutting, hauling, and storage

in the green state prior to dehydration, little decrease in the all-trans isomer occurs. When the plants are heated, however, this form decreases rapidly. Laboratory results have confirmed these findings. Since carotene is destroyed by oxidation during storage, several methods of preservation are now under consideration by industrial and research workers. Refrigerated warehousing, packing in sealed containers with removal of air or replacement of air by an inert gas, and the use of chemical antioxidants are effective in various degrees as stabilization measures.

New Products Tested for Biological Action

Conalbumin, a protein constituent of egg white, and siderophilin, a minor protein constituent of blood plasma, are similar in that both are iron-binding proteins. Moreover, they cannot be differentiated by chemical and physical-chemical criteria. Siderophilin serves the important function of transporting iron, an essential element in blood, to the sites of hemoglobin formation, and has therapeutic value for treating certain disorders of the circulatory system. Since adequate quantities of siderophilin are not available, the similarity between conalbumin and siderophilin suggested that the former, which is more readily available, might be used therapeutically in place of the latter.

To determine whether it would be safe to use conalbumin as a therapeutic agent, the Pharmacology Laboratory studied the physiological effects of this protein in comparison with those of siderophilin on laboratory animals. When tried on rabbits, the highly specific immunological reactions, which result in a precipitate or cloudiness of serum when an antigen combines with an antibody, showed that conalbumin and siderophilin are separate and distinct entities. Unfortunately, therefore, conalbumin, which could be made available in substantial quantity, cannot be used therapeutically in the place of siderophilin.

Xylose, a sugar having five carbon atoms in its molecule, is one of the byproducts formed when the cellulose in crop residues is converted to fermentable sugar by acid hydrolysis. Since xylose is not assimilated by human beings, it might be used in reducing diets and in food for diabetic patients, provided it were nontoxic. It had been reported to produce cataract in rats when it constituted 35 percent of the diet. This report was confirmed. However since 35 percent xylose is far above the concentration likely to be used in diets under practical conditions, investigations were started to determine the minimum concentration of xylose that will produce cataract. This work is still in progress, but thus far it has been found that a concentration of 15-percent xylose produces cataracts that tend to disappear as the experimental animals mature.

Pharmacological investigations were continued to learn why and how rutin acts as a vitimin (vitamin P). The sparing action of rutin toward ascorbic acid (vitamin C) was shown to prolong the life of scorbutic guinea pigs given insufficient ascorbic acid to cure them. When guinea pigs were given doses of ascorbic acid in excess of the curative dose, the administration of rutin increased the quantity of ascorbic acid stored in the adrenal glands. These observations suggested that rutin is a useful adjunct to ascorbic acid in the treatment of scurvy.

A report by other investigators that rutin exerts a beneficial action against experimental frostbite in rabbits was verified. As a part of the investigation to determine the relationship of rutin's chemical structure to its physiological action, the ability of another flavone glycoside, quercitrin, and its aglycone, quercetin, to reduce the severity of frostbite was studied. Since these two compounds are about as effective as rutin, it may be concluded that the rhamnose and glucose parts of rutin are not essential for the physiological effects.

Studies were made on the antioxidant action toward epinephrine of rutin and 17 other flavonoids of different, but related, chemical structure. The data indicated that the antioxidant phase of vitamin P activity is dependent upon a double bond, between C–2 and C–3, and two free hydroxyl groups, ortho to each other, on the phenyl ring.

Microscopical examinations of damaged tissues of the animals used in chronic toxicity tests of agents used to inhibit biological processes in seeds [ethylene chlorohydrin, propylene gylcol dipropionate, vinyl propionate and 1,3-dimethyl–4–6–bis (chloromethyl) benzene] were completed. In no case was a significant change noted that could be attributed to the compound fed. The results confirm the lack of chronic toxicity as judged by other criteria when dosages likely to be encountered under practical conditions are used. The conclusions apply only to chronic toxicity and do not rule out the possibility of acute skin-irritant effects.

Enzyme Studies Reveal Action of Phosphate Insecticides

Several new and very efficient insecticides were developed during and after World War II. Initially this development resulted from an attempt to find new poison gases and also substitutes for nicotine. Of particular interest are the new insecticides belonging to the class of chemicals known as esters of phosphoric acid, some of which are very powerful poisons. One of the simplest and most poisonous substances of this class is di-isopropyl fluorophosphate. This compound was used by the Enzyme Research Division as a simple model to study the reasons why such substances are poisonous.

It was already known that a reaction takes place between di-isopropyl fluorophosphate and a vital constituent of nerve tissue whereby the nerve tissue becomes paralyzed. Recently it was found that this poison also reacts with other proteins found in both plant and animal tissues. For example, certain proteins in citrus fruit combine with it.

It was also found that di-isopropyl flourophosphate combines readily with one vitally important enzyme protein in the animal body that can be obtained in the pure state. The enzyme in question (chymotrypsin) is one of the digestive enzymes derived from the pancreas. This reaction was therefore studied in detail, and the end product of the reaction—that is, the protein after it has been altered by the poison—was also prepared in pure, crystalline form. Analysis of this altered protein revealed that the fluorine of the di-isopropyl fluorophosphate had been given off as hydrofluoric acid, while the di-isopropyl phosphate radical combined with the enzyme protein and became part of it. Only one molecule of di-isopropyl fluorophosphate was found to combine with one molecule of the enzyme protein, but this slight alteration in the structure of the enzyme was sufficient to inactivate it completely as far as known tests can show.

Thus in one instance the reaction of a toxic phosphate and an enzyme protein is definitely known. There are reasons for believing that the same type of reaction takes place in nerve tissue, and causes the nerves to be paralyzed by the poison. A reaction similar to the one first described has also been observed in this laboratory with other enzymes of the digestive tract. Thus it appears that the poison, while able to combine with and destroy many enzymes of the body, probably combines with the vitally important nerve enzyme by preference. The understanding of this reaction is the first step in devising an adequate antidote. It also opens the way for the development of other substances of similar nature, but more suitable for their intended purposes—whether as war gases or as insecticides. Probably such modification of the poisonous properties of these substances can be achieved by changing their composition slightly. For example, it is known that diethyl fluorophosphate resembles di-isopropyl fluorophosphate chemically but is nevertheless less poisonous. Furthermore, the substitution of chlorine or bromine for fluorine in fluorophosphates greatly reduces the toxicity of the substance produced. Obviously it is possible to prepare a great many substances of this nature and to test out their properties.

Antibiotics Found in Higher Plants

Under a Research and Marketing Act project, the Bureau's Biologically Active Compounds Division continued its research on antibiotics from agricultural sources and found that some plants are potentially rich sources of antibiotics having a wide range of selectivity and specificity toward micro-organisms. Extracts of about 100 different plants had been tested previously for antibiotic activity, and during the past year some of the more promising plants were investigated further.

Sweetpotato Plant.—One variety of sweetpotato plant was found to be resistant to *Fusarium* wilt, whereas another variety was found to be susceptible to this disease. No marked differences were found between direct water extracts of the dried resistant and susceptible plants or individual parts of them when tested for their effects upon cultures of the fungus causing wilt and cultures of pathogenic bacteria of three types. The extracts of entire plants had little or no inhibiting effect. Instead, they showed a stimulating effect on the growth of three of the test micro-organisms. In marked contrast, the water-soluble portions of the methanol extracts of the dried plants or plant parts of resistant and susceptible varieties of sweetpotato inhibited cultures of micro-organisms previously unaffected or stimulated in some degree.

Efforts to recover and fractionate the antibiotic substances in the sweetpotato plant were concentrated on the extracts from the stems of the wilt-susceptible variety, because they appeared to be more active than extracts of the entire plants or other plant parts. The water-insoluble portion of the methanol extract from stems of susceptible plants showed strong antifungal activity toward two fungi that cause wilt in tomatoes and sweetpotatoes but no activity against Gram-positive, Gram-negative, and acid-fast bacteria. On the other hand, the water-soluble portion of the same extract was very active against Gram-positive and acid-fast bacteria but less active toward the fungi

and Gram-negative bacteria. When a concentrated solution of this water-soluble portion of the methanol extract was passed through a chromatographic column, the substance that passed through without being adsorbed stimulated the growth of the two fungi, inhibited the growth of two Gram-positive and two Gram-negative bacteria, and had no effect on another Gram-positive and a Gram-positive and acid-fast bacterium. A solution of the substance adsorbed by the chromatographic column inhibited the growth of three Gram-positive bacteria, the Gram-positive and acid-fast bacterium, and one Gram-negative bacterium but had no effect on the two fungi or another Gram-negative bacterium.

From the results obtained thus far it appears that the sweetpotato plant contains at least three distinguishable antibiotic components. Fractions containing them have been further purified and preliminary tests of their toxicities are under way.

Cabbage.—A crystalline substance isolated from a methanol extract of dried cabbage was found to have antifungal activity but no antibacterial activity. It has a melting point of 74° to 75° C., is soluble in most organic solvents, is sparingly soluble in hot and cold water, and gives negative tests for carbohydrates, aldehydes, and phosphorus. Analysis of the infrared spectrum of this compound indicated a fairly simple straight-chain molecule with a carbonyl or carboxyl group such as would be found in a ketone, ester, or acid. These results, together with those obtained from determinations of carbon, hydrogen, and oxygen, suggest that the compound may be 15-nonacosanone.

Another antifungal fraction was obtained when the methanol-soluble residue was extracted with 95-percent ethanol. On the basis of its activity and chemical reactions, this 95-percent ethanol-soluble fraction does not appear to be the same as the crystalline compound obtained from the methanol extract. When the 95-percent ethanol-insoluble residue was extracted with diethyl ether a fraction active against fungi and Gram-positive, Gram-negative, and acid-fast bacteria was obtained. The active substance was soluble in carbon tetrachloride, petroleum ether, chloroform, and acetone and gave positive phenol and Molisch tests and a negative carbonyl test.

Both fractions are being purified and characterized.

Bananas.—The banana skin has been referred to as nature's bacteriaproof wrapper, and it is possible that the presence of antibiotic substances in the skin is responsible for some of its protective qualities. Antibiotic tests have been made on the water-soluble fraction of methanol extracts from the pulp and from the skins of green, naturally ripened, and ethylene ripened bananas. Antifungal activity was exhibited by all six extracts. There was very little if any measurable antibacterial activity in either the pulp or skins of green bananas, but there was appreciable antibacterial activity in the pulp and skins of ripe bananas. The results showed definitely that the resistance of bananas to bacteria is due to the formation of inhibitory compounds during ripening.

The water-soluble portion of a methanol extract of ripe banana skins was found to inhibit the growth of three pathogenic fungi. Low-pressure distillation of a methanol concentrate of ripe banana skins produced a small quantity of liquid. This liquid was found to possess strong antibiotic properties toward fungi and toward Gram-positive, Gram-negative, and acid-fast bacteria. Some crystalline ma-

terial was obtained from the distillate, and this is being characterized.

SUMMARY.—The results of the investigations on these three plants show that there are probably eight distinct antibiotics present. From the cabbage plant one pure, crystalline, antifungal agent was isolated; and a second antifungal fraction and a third fraction that is active against Gram-positive, Gram-negative, and acid-fast bacteria were separated and purified. Extracts from the sweetpotato plant were fractionated into three parts; one was active against fungi, another primarily active against Gram-negative bacteria, and a third against Gram-positive and acid-fast bacteria. Green bananas were found to contain antifungal agents, whereas ripe bananas were shown to contain both antifungal and antibacterial components. Some human diseases, such as systemic fungus infections, tuberculosis, and virus infections, are not effectively controlled or cured by known antibiotics. In view of this situation and the promising results obtained on the specific antibiotic activity of extracts and compounds from these edible food products, further investigations seem to be warranted.

Tomatine and Tomatidine Characterized

More than 400 grams of crystalline tomatine was prepared from dried tomato leaves by the Biologically Active Compounds Division, using methods outlined in last year's report. Two hundred grams of this crystalline compound was recrystallized five times successively with recovery of 100 grams of purified tomatine, which was used for investigations on the chemical character of this compound. About 50 grams of crystalline tomatidine, the alkaloid portion of the complex tomatine compound, was prepared for a similar purpose. The possible importance of tomatine and tomatidine in making tomato plants resistant to disease was explored further, as well as the possible use of these compounds as antifungal therapeutic agents in the treatment of certain insidious diseases of man that result from fungus infections. Conclusive results have not yet been obtained in these biological investigations.

Sugars in tomatine identified

Tomatine has been characterized as a glycoside comprising alkaloid and carbohydrate fractions. When tomatine is treated with acids the molecule splits into tomatidine and a carbohydrate fraction composed of possibly four different sugars. A paper-partition chromatographic apparatus was developed with which a separation of the carbohydrate fraction into its component parts was achieved. Chemical tests applied to these sugars in comparison with known sugars as standards established the identity of three of them as xylose, glucose, and galactose, leaving one sugar yet unidentified.

Tomatidine found in all parts of tomato plants

Dried leaves, stems, and roots of Red Current, Pan America, Marglobe, Rutgers, and Bonny Best tomato plants were extracted to learn how tomatine and tomatidine are distributed in different varieties and different parts of tomato plants. Since tomatidine is the easier to isolate, the crude tomatine fraction from each of the plant parts of different varieties was treated with hydrochloric acid, and the tomatidine hydrochloride formed was isolated and converted to the free base. A few milligrams of tomatidine was obtained from each of the

plant parts from different varieties of tomato. Preliminary investigations on the tomatidine fractions isolated from the various plant parts and from the different varieties showed that their infrared absorption curves were identical. These results indicated that tomatidine occurs throughout the plant and is present in each of the tomato varieties examined. The isolation of tomatidine from other species of tomato plants is being continued to determine whether this same substance is produced uniformly by all species and varieties of tomato plants, since the results may have a direct bearing on the disease resistance of the tomato varieties.

Tomatine found to have growth-regulating activity

If tomatine, an antifungal agent isolated in crystalline form from the tomato plant, is at all responsible for the resistance of tomato plants to *Fusarium* wilt (a fungus disease), it would obviously have to be transportable within the plant. Recently it was found that tomatine or tomatidine occurs in all parts of the tomato plant, and from earlier experiments it was tentatively concluded that when a tomato plant wilts its tomatine content disappears. During the past year it was demonstrated through cooperative work with the Bureau of Plant Industry, Soils, and Agricultural Engineering that tomatine is capable of being translocated in a plant and also that it appears to have a growth-regulating effect on bean seedlings of the kind used for testing plant-growth regulators.

Tomatine brought about formative effects on the first trifoliate leaf of bean plants when applied to the first internode in a small area ringed with lanolin. However the first trifoliate leaf was the only one on each plant to show the formative effect. Terminal growth of treated plants was reduced 24 percent as compared with that of untreated controls.

Tomatidine hydrochloride, when tested in a similar manner, was found to be toxic to cells near the treated area. The symptoms were confined to the treated part of the stem. Reduction of the terminal growth was insignificant in all tests with this compound. There was no evidence that the compound was translocated to the terminal bud of the bean plant.

Tomatidine, the alkaloid portion of tomatine, was toxic in a manner similar to that of tomatidine hydrochloride, although the injurious effects were more severe. No evidence was obtained that this compound was translocated to the terminal bud of the bean plant.

It is significant that only when the sugar portion of the tomatine molecule remained attached to the alkaloid was there any evidence of translocation of the latter. The toxicity and possible injurious effect of tomatidine in the tomato plant must now be considered, since the *Fusarium* wilt fungus may produce an enzyme that is capable of splitting tomatine into tomatidine and sugar portions. This work is being continued in order to properly evaluate the significance of these findings in plant-disease investigations.

Lupulon and Humulon of Hops Studied as Antibiotics

Lupulon and humulon, two constituents of hops that are important in brewing are under investigation as antibiotics at the Western Regional Research Laboratory. Although their bacteriostatic potency has been recognized for many years in connection with brewing,

no previous tests had been made of antibiotic activity toward animal or human pathogens. Detailed antibiotic tests with the pure compounds showed moderate to high activity toward Gram-positive and acid-fast bacteria. Lupulon is about 10 times as active as humulon. The substances are inactive toward Gram-negative bacteria, and have only slight activity toward yeasts, other fungi, and actinomycetes.

A simple method for the isolation of lupulon, first used in 1886, was adapted to large-scale recovery. It involves extraction of hops with petroleum ether and direct crystallization of lupulon from a vacuum concentrate of the extract. Humulon can be recovered from such a concentrate, before or after removal of lupulon, by precipitation with *o*-phenylenediamine. The yields obtained were up to 1.5 percent for lupulon and up to 4 percent for humulon salt.

Collaborative investigations by the University of California Medical School showed that lupulon has tuberculostatic activity both in vitro and in mice. Tuberculosis-infected mice, after daily treatment intramuscularly or orally for 3 weeks with lupulon, had substantially reduced numbers of lesions and of acid-fast bacteria in the lesions. Confirmatory experiments with mice and guinea pigs are underway at several research institutions.

In view of the relatively low toxicity of lupulon toward mice and other animals, a large supply (more than 20 pounds) of lupulon has been prepared for chronic toxicity tests with animals and for preliminary tolerance and therapeutic trials with human beings. Preliminary experience indicates that oral therapy is accompanied by disturbances in the gastrointestinal tract, which may be severe. The results appeared to warrant trials in which lupulon will be administered intramuscularly. The ultraviolet spectrophotometric assay method for lupulon described on page 84 of this report is being adapted to the assay of lupulon in blood serum, so that pharmacological studies on absorption of the drug can be made.

Since the humulon and lupulon in hops are broken down in the process of boiling the wort, it appears that the antibiotic activity in beer is due to their degradation products. Work is in progress on the laboratory production, isolation, and identification of these breakdown products for the purpose of evaluating their antibiotic activity.

Experimentally produced samples of lupulon and humulon have been distributed to many pharmaceutical houses and research scientists. The compounds also have been made available commercially by a small chemical supply company.

Natural and Synthetic Plant-Growth Regulators Studied

The need for better and more specific chemical weed killers and other plant-growth regulators continues to emphasize the necessity for a better knowledge of how these compounds bring about changes in plants. Since there is a delicate balance of natural regulators in all plants, it seems possible that new types of regulators may be found in plants themselves or in plant parts.

Growth regulators obtained from agricultural sources

A new line of research aimed at the isolation, identification, and characterization of plant-growth regulators in agricultural products was initiated during the past fiscal year under a Research and Mar-

keting Act project. This work is being done by the Biologically Active Compounds Division with the cooperation of the Bureau of Plant Industry, Soils, and Agricultural Engineering which conducts the assays and evaluates the potency of products isolated or purified.

Corn pollen is one of the agricultural products under investigation as a source of natural plant-growth regulators. Optimum conditions for the extraction and preparation of active concentrates were worked out, and purification of these concentrates by use of chromatographic and distribution techniques yielded active fractions and two crystalline compounds. However the two crystalline compounds failed to show the marked stimulatory activity of the original pollen extract. The active principle retained its activity after being heated at 80° to 85° C. for 3 hours while suspended in water; when heated similarly in *N* sodium hydroxide solution its activity was slightly depressed; but heating in *N* hydrochloric acid solution completely destroyed its activity. Experimental results indicated the possible presence of two plant-growth regulators. One causes the entire bean plant to bend from the ground level when applied at any internode, whereas the other causes a stimulation of growth (evidenced by bending) at the internode only.

The possibility that corn pollen contains indoleacetic acid was examined critically. The best purified fractions obtained were found to stimulate young bean plants more than did the optimum concentration of indoleacetic acid. In line with this biological evidence, chemical tests for the presence of indoleacetic acid in corn-pollen extracts were negative. For testing the presence of indoleacetic acid, a paper-spot test was developed.

Distiller's "solubles," a byproduct of alcohol manufacture from grain, was found to contain very active plant-growth regulating substances. By use of counter-current-distribution procedures it was possible to demonstrate the presence of more than one growth-regulating substance, one being a growth stimulator that might or might not act as a growth inhibitor in higher concentrations, and another being a substance that kills plants without having any growth stimulating effect and might therefore be considered a plant poison.

The isolation and identification of naturally occurring growth regulators should lead to a better understanding of the chemistry of plants and plant products and might indicate the type of synthetic compounds that should be synthesized to obtain a specific and desirable effect in the solution of certain agricultural problems.

Radioactivity aids growth-regulator research

The synthesis of a new plant-growth regulator, 2,4-dichloro-5-iodophenoxyacetic acid, was announced in last year's report. Twenty millicuries of radioactive iodine was used in synthesizing 18 grams and imparted a low degree of radioactivity to the compound and 11 derivatives (ammonium, ethylammonium, diethylammonium, triethanolamine, sodium, and calcium salts; an acid chloride; an amide; and methyl, isopropyl, and *n*-butyl esters). During the past year, in cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering, these compounds, labeled with radioactivity, were applied to different plants, and their distribution in the plants was determined. They were found to accumulate primarily in the newest tissues at growing points.

The new regulator, 2,4-dichloro-5-iodophenoxyacetic acid, is closely related to the well-known weed killer, 2,4-dichlorophenoxyacetic acid, and although its plant-growth regulating activity is only about 60 percent of the latter, due to the substitution of iodine for hydrogen in the benzene ring, the kind of plant response to it is quite similar. The esters and amide derived from the regulator containing radioactive iodine had greater regulating activity than their parent acid when tested on an equivalent-weight basis, whereas the salts had lower or no better activity than their parent acid. The growth-regulating activity of some esters approached but in no case equalled that of 2,4-dichlorophenoxyacetic acid. Complete evaluation of the results obtained with such compounds should add to the present meager knowledge of the mechanism of action of plant-growth regulators. The work on radioactive compounds was supported by an allotment from the special research fund authorized by the Bankhead-Jones Act of June 29, 1935.

New synthetic compounds tested for growth-regulating properties

More than 150 new synthetic compounds were prepared as possible plant-growth regulators and are being tested in cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering. These compounds include substituted amino acids, phenyl, phenoxy, pyrimidine, naphthalene, pyridine, quinoline, and mercapto compounds. An attempt to prepare one new plant-growth regulator, alpha-naphthalene butynoic acid, resulted in the synthesis of two isomers. The molecular configuration of each is being determined. One isomer has a melting point of 112° C. and is very active as a plant-growth regulator, whereas the other melts at 127° C. and is much less active. The activity of the very active isomer is somewhat similar to that of alpha-naphthalene acetic acid which promotes rooting and gall formation. This is the first aromatic-acetylenic acid to be tested as a plant-growth regulator, but other compounds of this general type are being synthesized.

Allergens Studied to Learn How Composition Is Related to Activity

The Bureau's research on the allergens of agricultural products, which is supported by an allotment from the special research found authorized by the Bankhead-Jones Act of June 29, 1935, started with a thorough investigation on the isolation and identification of the allergens of cottonseed. The active substance in the most concentrated allergenic fraction prepared from cottonseed meal, designated as "Cottonseed–1A" in several publications on the subject, was a complex compound of protein and polysaccharide, belonging to the class of nitrogen compounds known as native proteoses (meaning protein-sugars).

Later, investigations were made on the allergens of castor-beans, flaxseed, filberts, and other oilseeds and nuts. In each case the allergen was separated from a complex mixture of seed proteins by a procedure similar to the one used for isolating the cottonseed allergen. The allergens from these sources were likewise found to be proteoses, and tests on guinea pigs with the allergens modified by removing different proportions of the polysaccharide led to the conclusion that the allergenic activity of these substances depends on their protein components. It was very desirable to learn how these proteins differ in their make-up from proteins that are not allergenic.

Oilseed allergens analyzed for amino acids

During the past year some progress toward the correlation of the chemical composition of allergens with their biological activity was made by quantitative determination of the amino acids in purified oilseed allergens. Chemical analyses were made of 11 allergens that had been isolated from oilseeds during the past several years. From 88 to 95 percent of the total nitrogen was accounted for in each case by the various amino acids found. Nitrogen found in the form of ammonia, which is produced by partial and unavoidable destruction of amino acids, accounted for most of the remaining nitrogen.

Important generalizations and some points of special significance were derived from a compilation of the analytical results. Eighteen of the twenty-two natural amino acids now known to biochemists were identified as components of the oilseed allergens, and these 18 amino acids were found in each of the allergens, excepting 1 from castor-beans and 1 from brazil nuts.

The allergenic fraction from castor-beans (castor bean–1A), which is the most potent allergen known, contained no tryptophan. The allergenic fraction from brazil nuts (brazil nut–1A) contained neither tryptophan nor threonin. In general the proportions of arginine and glutamic acid found in the allergens are relatively higher than found in most other proteins, except that some protamines contain more arginine—almost 90 percent. The 1A fraction from Duchilly filberts contained the most arginine, with 40.1 percent of its nitrogen in that form. This allergen also had the highest total arginine and glutamic acid content with 60.5 percent of its nitrogen in these two amino acids. Cystine nitrogen was relatively higher in these allergens than in most proteins. Brazil nut fraction 1A contained 5.2 percent of its nitrogen in the form of methionine, which was unusually high and more than twice as much as was found in any of the other allergens. Castor-bean fraction 1A contained 9.1 percent of its nitrogen as serine—almost three times more than was found in any other allergen.

Generally the allergens were poor in those amino acids containing the aromatic nucleus, that is, tryptophan, tyrosine, and beta-phenylalanine. This evidence is pertinent to the correlation of chemical composition with immunologic activity, since other investigators have advanced the theory that to be antigenic a protein must have an amino acid containing an aromatic nucleus. All of the allergens of this group contained two or more of these amino acids, but in notably small proportions. Also relevant to the relation between composition and antigenic specificity is the close similarity in amino acid make-up of the allergens obtained from two varieties of filberts, and the fact that these two allergens were immunologically identical.

The results obtained in this study of oilseed allergens support the concept that these allergens comprise a separate class of native proteins. They resemble proteoses, long recognized as derived proteins, with respect to chemical and physical properties, including diffusibility in dialytic systems, but they differ from all derived proteins in possessing the antigenic activity of native proteins.

As rapidly as possible the immunologic properties of available 1A fractions of oilseeds are being studied. During the past year the antigenic activity of 1A fractions from almonds, brazil nuts, and flaxseed were investigated. The immunologic properties of these 1A fractions were typical of those represented by cottonseed 1A and

castor-bean 1A. Each fraction was shown to contain preformed protein native to the respective seed and of molecular size that permitted rapid diffusion through semipermeable membranes. Dialyzates from almonds and brazil nuts contained only antigens identified with the 1A fraction. An unidentified, diffusible antigen, distinguished from flaxseed 1A, was detected in the dialyzate from a water-extract of flaxseed.

Study made of factors affecting anaphylaxis

Fundamental studies of active anaphylaxis in guinea pigs were continued this past year with the object of developing better methods for the quantitative determination of antigenic capacity of allergenic proteins. Such quantitative measurements are needed to correlate antibody production with systemic sensitivity and with anaphylactic sensitivity in excised smooth muscles. These studies, though not completed, have already provided important practical information for quantitative determination of antigenic characteristics of allergens and, also, for correlation of chemical and immunological properties of certain diagnostic serums.

The data obtained during the past year confirmed earlier preliminary results, which indicated that (1) sensitization of guinea pigs with alum-precipitated antigen enhances antibody formation and sustains antibody production at a higher level over a longer period of time than sensitization with untreated antigen, (2) sensitivity in excised smooth muscle tissue is directly proportional to the antibody concentration of the blood serum, and (3) systemic sensitivity is indirectly proportional to the concentration of serum antibodies.

Progress Made in Fractionating Allergens of Johnin and Tuberculin

Further progress was made during the past year in the fractionation of johnin and tuberculin in conjunction with an immunologic study of these bacterial products. This investigation, which is supported by an allotment from the special research fund authorized by the Bankhead-Jones Act of June 29, 1935, was started in 1946 to explain why false positive skin reactions are sometimes obtained with johnin or tuberculin, by veterinarians in testing livestock for Johne's disease or tuberculosis, respectively, leading to uncertain or incorrect diagnosis. The discovery that johnin and tuberculin contain allergens that induce anaphylaxis in guinea pigs made possible a new approach to the solution of the problem of distinguishing Johne's disease from tuberculosis at an early stage of infection.

Antigen of tuberculin concentrated by acid precipitation

The most promising of several fractionation procedures reported last year was the concentration of tuberculin-active components obtained by acidifying the unconcentrated filtrate from a culture of human tubercle bacillus with hydrochloric acid. Experiments on this general scheme of fractionation were continued to establish more precisely the most favorable conditions and procedures for concentrating either or both homologous and heterologous antigens of tuberculin. These experiments confirmed the previously reported observation that the unconcentrated culture filtrate was superior to the usual tenfold concentrated filtrate for fractionation with hydrochloric acid.

Best results were obtained by fractionating the total acid-formed

precipitate (refined by five reprecipitations under the same conditions) with hydrochloric acid under different conditions designed to follow the distribution of the homologous and heterologous antigens at various pH values. Selected fractions were evaluated by bovine skin tests in comparison with the standard tuberculin.

In a series of successive precipitations at hydrogen-ion concentrations ranging from pH 5 to pH 3.3, the first fraction that showed, to a highly significant degree, both an increase in potency of the homologous (tuberculin) antigen and a decrease in the heterologous (johnin) antigen was precipitated at pH 4.7. A fraction obtained at pH 5.1 also showed significantly higher homologous and lower heterologous activity in comparisons with the standard tuberculin. Thus the most favorable conditions of acidity established by this series of fractionations were within the range of hydrochloric acid concentration represented by pH values of 5.1 and 4.7. An experiment was started to determine whether a practical procedure could be devised for further separation of the skin-reactive antigens by graded precipitation with hydrochloric acid within this range. Incomplete results of bovine skin tests with fractions from this experiment indicated that graded hydrochloric acid precipitation of the total acid-formed precipitate from an unconcentrated culture filtrate of human tubercle bacillus is a feasible procedure for significant improvement in specificity of the skin-reactive tuberculin antigen.

Comparison made of tuberculins from human and bovine tubercle bacilli

Tuberculin produced by the Bureau of Animal Industry for detecting tuberculosis in cattle is derived from the bacillus of human tuberculosis rather than from that of the bovine type because of advantages inherent in cultural characteristics of the former. Theoretically the culture filtrate of bovine tubercle bacillus might yield by fractionation a tuberculin of superior specificity and potency for diagnosis of bovine tuberculosis. During the past year the availability of filtrate from bovine tubercle bacillus cultures in sufficient quantity for fractionation prompted an exploratory comparison of this filtrate with the culture filtrate from human tubercle bacillus. Accordingly an unconcentrated culture filtrate from bovine tubercle bacillus was acidified with hydrochloric acid to pH 4.95. The precipitate that formed settled completely, leaving a clear supernatant solution. This precipitate was separated, and another fractional precipitate was obtained from the supernatant solution by acidification to pH 3.5. After separation of this precipitate, alcohol was added to the supernatant portion to 90-percent concentration to obtain a third precipitate. The original filtrate from bovine tubercle bacillus culture and the three fractions separated from it were evaluated by bovine skin tests in comparison with a fraction precipitated at pH 5 from the conventional tuberculin prepared from the filtrate of a culture of tubercle bacillus of the human type.

Even within the limited range of fractionation acidities employed in this experiment there was evidence of significant concentration of homologous skin-reactive components of the culture filtrate from bovine tubercle bacillus. However, the fractions separated from bovine culture filtrate contained a noticeably small proportion of the nitrogen of the original filtrate. All three fractions accounted for

only 12 percent of the nitrogen in the unconcentrated culture filtrate. On the other hand, 26.5 percent of the nitrogen in the unconcentrated filtrate from a culture of tubercle bacillus of the human type was precipitated by acidification to pH 5.

The low proportion of nitrogen precipitated from the original bovine filtrate in the fraction of highest activity might indicate a significant difference in the chemical compositions of the acid-precipitable components of the two types of culture filtrates. To test this possibility, the two fractions that respectively represented the highest concentration of skin-reactive components from the two types of culture filtrates were analyzed for distribution of amino acid nitrogen. The two fractions were similar with respect to the proportions of methionine, glutamic acid, arginine, and histidine. Determinations of the remaining amino acids were not completed before the end of the year. Further fractionation of culture filtrates from bovine tubercle bacillus will be required to determine whether the possible advantages to be gained in potency and specificity of these fractionation products would compensate for the disadvantage of slow growth rate of the culture.

Reasons sought for lack of agreement between anaphylactic response of guinea pigs and bovine skin tests

The fractionation of tuberculin and johnin to isolate the diagnostic, skin-reactive antigens depends on having a reliable method for evaluating potency and specificity of each fraction. Bovine skin tests provide the final criterion of diagnostic specificity, but skin tests on cows become prohibitive in cost when employed for evaluating the numerous products of progressive fractionation. Accordingly an investigation of the anaphylactic antigens of tuberculin and johnin was undertaken to develop tests on guinea pigs that would provide the essential guidance for the fractionation procedures.

Use of the anapyhlactic response of the guinea pig to evaluate the antigens that affect bovine skin was predicated on the hypothesis that both the skin reaction of the tuberculin- or johnin-sensitized cow and the anaphylactic reaction of the guinea pig, sensitized with the same product, were induced by the same protein components of tuberculin or johnin and, further, that the specificity of the functional antigen in the two reactions would be determined by the same reactive groups. This hypothesis appeared to be confirmed by the observations that crossed reactions of johnin and tuberculin in the anaphylactic tests paralleled crossed reactions induced by the same preparations in skin tests on sensitized cows. However, discrepancies appeared in the results obtained in testing certain of the fractionation products of tuberculin and johnin by the two methods. These discrepancies made doubtful the original interpretation of the similar crossed reactions observed in anaphylactic and bovine skin tests. Therefore the study of these immunologic reactions was continued to determine the cause of discrepancies in the values obtained in testing the fractionation products by their anaphylactic and skin-reactive properties.

It was believed that possible reasons for the discordant results might be found in (1) differences in response of different animal species to sensitization with the same antigen, (2) differences in antigens that can sensitize the skin of the cow and the uterine muscle of the guinea pig, (3) differences in the effect on different animals or organs of

adjuvants used to enhance sensitizing capacity, and (4) modification of the antigen by agents or conditions of fractionation with resulting different or variable effects on anapyhlactic and skin-reactive functions.

To induce the tuberculin type of skin sensitivity in guinea pigs, infection with living tubercle bacilli or injection of dead tubercle bacilli is necessary. On the other hand, injection of cell-free tuberculin will induce anaphylactic sensitization. Successful sensitization of guinea pigs with either agent requires an adjuvant, such as alum, to enhance the sensitizing capacity of the antigen. Guinea pigs are sensitized for anaphylactic tests with alum-precipitated human-type tuberculin injected subcutaneously, whereas cows are sensitized for skin tests with dead bovine tubercle bacilli suspended in mineral oil and injected intraperitoneally. Hence the procedure used to sensitize cows for skin tests differ from that used to sensitize guinea pigs for anaphylactic tests.

There is certainly some possibility that the fractionation procedures used to concentrate antigens caused some degradation of the active principles of tuberculin and johnin. Such degradation could diminish the protein's anaphylactogenic properties to a greater degree than its skin-reactive properties. This effect would correspond with previously reported observations on the comparative effects of degradative agents on the anaphylactic properties and the skin-reactive properties of the cottonseed allergen.

In one method of investigating the reason for the discordant results, a lot of guinea pigs was equally divided into groups 1, 2, and 3 which were sensitized, respectively, with mineral-oil emulsions of (1) dead bovine tubercle bacilli, (2) bovine tuberculin, and (3) a combination of dead bovine bacilli and bovine tuberculin. Sensitization of the third group with the combined antigens was prompted by published reports that dead tubercle bacilli enhance the sensitizing potency of the antigens.

A second lot of guinea pigs was likewise divided into three groups which were sensitized, according to the same plan, with alum-precipitated preparations of the same three antigens.

Half of the animals in each group were sacrificed, and their uterine muscles were tested for anaphylactic sensitivity to human tuberculin, bovine tuberculin, and johnin by the Schultz-Dale method. The remaining animals were first tested for skin sensitivity to the same three antigens and then sacrificed for Schultz-Dale tests of anaphylactic sensitivity.

These experiments yielded some interesting preliminary results. Animals sensitized with oil-emulsified dead cells of bovine tubercle bacillus showed uniformly high skin sensitivity to human and bovine tuberculin and to johnin. By contrast, the anaphylactic responses were variable; some showed a high degree of sensitivity, and others ranged from moderate to negligible reactions.

Animals sensitized with oil-emulsified bovine tuberculin showed low skin sensitivity and uniformly moderate anaphylactic sensitivity.

Animals sensitized with oil-emulsified combination of bovine tuberculin and dead bacilli showed uniformly high skin sensitivity and moderate, but erratic, anaphylactic sensitivity. There was no evidence that the combination of dead tubercle bacilli and bovine tuberculin induced any greater sensitivity than either preparation alone.

Widest variability in sensitivity was encountered among animals sensitized with alum-precipitated antigens. Only 1 out of 11 animals sensitized with alum-precipitated, dead bacilli showed anaphylactic sensitivity. Skin sensitivity of all animals of this group was significantly lower than among those sensitized with oil-emulsified bacilli.

Alum-precipitated tuberculin induced moderate anaphylactic sensitivity, but a low degree of skin sensitivity.

Animals sensitized with the alum-precipitated combination of antigens showed insignificant skin sensitivity and negligible anaphylactic response. These results show that the alum-precipitated, dead tubercle bacilli may depress rather than enhance the sensitizing capacity of tuberculin.

There was some difficulty in using oil emulsions of the antigens owing to high viscosity of these preparations and consequent resistance to passage through hypodermic needles. Regardless of this difficulty the results reported here indicate that oil is superior to alum as an adjuvant for sensitization of guinea pigs. Moreover the uniform and high degree of skin sensitiveness obtained with oil emulsions of the dead bovine bacilli provides a new lead to the use of guinea pigs for evaluating fractionation products of tuberculin and johnin by skin tests similar to the conventional bovine skin tests.

RESEARCH INSTRUMENTS AND METHODS

New Instruments Developed for Measuring Large Molecules

The physical properties of industrial products derived from agricultural raw materials are dependent to a large extent on the molecular weights of the compounds utilized. This is especially true of polymerized products, such as plastics, synthetic rubbers, fibers, and films, derived from natural compounds such as starch, pectin, proteins, and fatty acid esters. The determination of high molecular weights is therefore a necessary step in the Bureau's research and development work.

At the Eastern Regional Research Laboratory a new instrument was developed to measure the molecular weight of polymers by optical means. It is known as a photoelectric light-scattering photometer, or an absolute turbidimeter. By measuring the light scattered by the dispersed particles in a dilute solution of a polymer it is possible, by application of Debye's theory, to determine the molecular weight (from 90° scattering), the particle size (from 45° and 135° scattering), and in some cases the particle shape (from depolarization of 90° scattering). The method is considerably faster than any previously used and yields more information regarding the molecules. It is also applicable to the determination of particle size in a suspension, such as natural or synthetic rubber latex.

Another optical instrument, called a differential refractometer, was developed by the Eastern Laboratory to determine the difference between the polymer solution and the solvent with regard to refractive index. This measurement is also required in the determination of molecular weight.

The new instruments were used for determining molecular weights of certain polymers as an aid to various investigations, including the following: Improvement of textile fibers from casein; fundamental

research on starch fractions: determination of useful properties of Lactoprene EV synthetic rubber; and studies on polymers made from fatty acid derivatives. The instruments are now available commercially and have proved to be acceptable to research workers. About 15 of them are in use in governmental, industrial, and university laboratories engaged in research on polymers.

Photochemical Oxidation Useful as New Tool for Protein and Virus Research

It was reported last year that nicotine is oxidized to a new compound in the presence of oxygen, methylene blue, and light. In further research the Eastern Regional Research Laboratory found that this photochemical reaction can be applied to tertiary amines in general, and is apparently different from any straight chemical oxidation known. Most amines, including nicotine, absorb oxygen, but no carbon dioxide is emitted. When the reaction is applied to amino acids, the rate of oxidation varies considerably, and carbon dioxide is evolved in some cases. For example, 1 mole of tryptophan absorbs 4 moles of oxygen and evolves 2 moles of carbon dioxide. It is not yet known what change takes place in the tryptophan molecule.

When a protein, such as beta-lactoglobulin, is photochemically oxidized the first phase of the reaction is confined to the tryptophan in the molecule. Thereafter the oxidation is slower, but it comes to a definite stop when about 40 moles of oxygen are absorbed.

Since tryptophan plays a specific role in many biologically active proteins, especially in viruses, cooperative preliminary trials were made with influenza virus by Children's Hospital of Philadelphia and with the virus of the Newcastle disease of poultry by the Bureau of Animal Industry. Again a rather definite amount of oxygen was absorbed, and both viruses were killed. The antigenic properties were also lost. It is possible that at some stage short of complete oxidation the virus may become attenuated in virulence and still retain its antigenic properties. This idea is being followed up.

Since both viruses were accompanied by a high proportion of non-virus protein and other substances, further attention is being centered on bacterial viruses, or phages, which can be prepared in highly purified form.

Hop Resin Assayed With Spectrophotometer

The petroleum ether-soluble fraction of hops, called "soft resin," is the main source of the flavor and preservative action contributed by hops in brewing. Humulon and lupulon are the primary constituents of the soft resin; of these the former is generally considered to be much the more important. Heretofore there has been no simple, rapid method for the determination of lupulon. The Association of Official Agricultural Chemists' method for the chemical analysis of hops called for determination of humulon gravimetrically as the lead salt; the difference between the total petroleum ether-extractables and the humulon was referred to as "beta resin." Because the official method for chemical hop analysis was tedious it never attained the general use in hop grading and certification as did physical determinations such as quantity of leaf-plus-stem and seed content.

During the past year it was discovered at the Western Regional Research Laboratory that the near ultraviolet absorption of humulon and lupulon is sufficiently intense, and the absorption of other constituents of petroleum-ether extracts of hops sufficiently low above wave lengths of 300 millimicrons (mμ), that humulon and lupulon can be determined in such extracts with a fair degree of accuracy. At 332 mμ the absorption coefficients of humulon and lupulon are identical, whereas at 355 mμ they differ markedly. The extract is diluted with alkaline methanol, and its optical density is read on the spectrophotometer at these wavelengths. The humulon and lupulon contents can be calculated from the absorption coefficients, or more conveniently by interpolation on a chart prepared from data obtained with solutions of known composition. The entire assay can be completed within 5 minutes; thus, for the first time a rapid and accurate method is available for the determination of these valuable hop ingredients.

This method has proved of great value in research at the Western Laboratory on improvement of methods for isolating humulon and lupulon and for evaluating the antibiotic activity of these substances.

Persons and firms interested in hop marketing, hop breeding, and brewing technology have shown considerable interest in the new assay method. Apparently it offers the possibility of much closer control of hop quality in marketing and brewing. In brewing research it will be useful for more precise evaluation of the roles of humulon and lupulon and for indicating the most desirable proportion of these substances in hops. In hop-breeding work it makes feasible the determination of humulon and lupulon in the large numbers of samples necessary to obtain genetic control of hops with regard to these constituents. In technological research on hop production, the method will permit determination of the influence of time of harvest, drying, and mode of storage on retention of humulon and lupulon.

Use of Pectin Guided by X-Ray Studies

In order to obtain maximum utilization of either a natural or synthetic fiber material it is essential to understand the molecular structure and packing arrangement of the high polymer chains making up the fiber. In particular, it is essential to know how the packing arrangement and configuration of the chains vary with elongation, chemical modification, and water content of the fiber. A great deal of information of this type is now available for some of the well-known fibers such as wool, cotton, and nylon.

Pectin is a material from which potentially important fibers and films can be made. In order to obtain information on the structure of the chains in fibers produced from pectin and chemically modified pectin, an X-ray diffraction investigation of pectin was undertaken at the Western Regional Research Laboratory.

The pectin molecule is essentially a long straight chain of polymerized galacturonic acid units. The X-ray diffraction investigation showed that the distance between geometrically identical positions along the chain is 13.1 Angstroms, whereas that for cellulose is 10.3 A. Since the monomers in cellulose and pectin have essentially the same size and it is known that cellulose contains two completely extended monomer units in the 10.3 A spacing, it follows that the identity period

in pectin must contain three monomer units. The monomer units in pectin, however, are inclined to the chain axis, so that the increment per monomer unit in the chain axis direction is only 4.37 A instead of 5.15 A as in cellulose. Because of this difference in inclination of the monomers to the chain axis the chains in cellulose are flat and pack together with considerable regularity, whereas the uneven chains in pectin tend to prevent a regular arrangement. As a result, cellulose is quite crystalline, whereas pectin is not. This difference in regularity of packing is partly responsible for the large observed difference in water-absorption capacities of pectin and cellulose.

Since the ability of pectin and its derivatives to absorb water and form gels is one of the important reasons why these materials have been used to make jams, jellies, and cold-process puddings, a more thorough understanding of the mechanism of water absorption was desired. From an X-ray diffraction study of sodium pectate it was found that the interchain separation in the crystalline portion increases when the water content increases from nil to about 24 percent but does not increase when the water content increases from 24 to 65 percent. The reason for this interesting behavior is that below a water content of 24 percent water is absorbed by both the crystalline and amorphous portions of the material, whereas above 24 percent only the amorphous part absorbs water.

As a result of these X-ray studies, the geometrical configuration and packing arrangement of the chains in pectin, as well as the mechanism of water absorption by pectin are now well understood. This basic information has led to increased understanding of the pectin molecule and has been of value in developing pectin films for coating candied fruits, figs, dates, and nuts.

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