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

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UNITED STATES DEPARTMENT OF AGRICULTURE

REPORT OF THE CHIEF OF THE BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY, AGRICULTURAL RESEARCH ADMINISTRATION, 1948

United States Department of Agriculture,
Washington, D. C., September 29, 1948.

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, 1948.

Sincerely,

Louis B. Howard, *Chief.*

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INTRODUCTION

The following pages report the progress that was made during the fiscal year 1948 in various research projects pursued by the Bureau of Agricultural and Industrial Chemistry. Most of the results reported are further contributions to the solution of problems that are subjects of long-time projects and have been mentioned in previous reports. Some are on new projects inaugurated during the year under the provisions of the Research and Marketing Act of 1946.

From August 1947 through June 1948 this Bureau was authorized to conduct independently, to supervise, or to participate in 37 RMA projects. The purpose of these projects is to expand existing, or hasten the development of new, commercial outlets for agricultural commodities. Some of the projects relate to commodities that were not receiving attention in the current research program of the Bureau,

namely rice and its byproducts, dried beans and peas, including splits and culls, honey and its byproducts, maple sirup and other maple products, wool, and the byproducts of wool scouring. However most of the RMA projects supplement work already done or in progress on certain agricultural commodities by providing for pilot-plant or experimental commercial production of new or improved products, and supplying special equipment or other facilities needed to promote the work.

Although the RMA projects have been in progress for less than a year, this report includes statements on 12 of them. These statements are not segregated, but are interspersed among statements on closely related work under the regular Bureau projects. They relate to cotton fibers, cottonseed, rice-bran oil, soybean oil, tobacco, plant sources of tanning extracts, fruit flavors, dried eggs, citrus-fruit products, plant sources of antibiotics, a new pine-gum product, and pear wastes.

The greater part of this report comprises brief statements, under informative headings, which point out the significance of results obtained by the four regional research laboratories on the industrial utilization of agricultural commodities of their respective regions. Then follow statements on the results of research bearing on industrial utilization of agricultural commodities that was accomplished by the Microbiology Research Division, the Fruit and Vegetable Chemistry Laboratory, the Enzyme Research Laboratory, the Agricultural Chemical Research Division, and the Naval Stores Research Division.

The last part of the report deals with some of the fundamental research of the Bureau which relates to agricultural commodities or their products and byproducts but is not limited to practical ends. It covers the work of the Biologically Active Compounds Division on antibiotics and plant-growth regulators, that of the Allergen Research Division on allergens, and some work of the regional research laboratories on protein, starch, and lignin.

As in past years, some of the Bureau's work on the utilization of agricultural products has attracted the interest of industrial firms. In some cases (A) products or processes developed by the Bureau on a laboratory or pilot-plant scale have been commercialized; in others (B) industrial firms are continuing the development work on a pilot-plant or semiworks scale preparatory to commercialization; and in still others (C) the published results of research or direct technical aid and advice to collaborators have enabled industrial firms to improve their processes or products.

Examples of (A) are: Rotproofing of ready-made cotton filter bags for water-softening systems by partial acetylation; extraction of rutin from green or dried buckwheat plants with hot isopropyl alcohol; production of *l*-ascorbyl palmitate under a Government-owned patent for use as a lard preservative; recovery of calcium aconitate from cane-sugar-mill molasses for use as a source of high-grade plasticizer; and cleaning pine gum by an improved process.

Examples of (B) are: Production of textile fibers from zein, the alcohol-soluble protein of corn; production of mold amylase by submerged culture for use as a malt substitute; manufacture of allyl starch as a resin replacement in liquid coatings and printing inks; and manufacture of the acrylic "rubber" Lactoprene EV, a copolymer of ethyl acrylate and chloroethyl vinyl ether.

Examples of (C) are: Commercial use of a constant-tension, tire-cord stretcher designed for making superior cotton tire cord; production of tanning extract from pecan shells; use of pectinate films for coating candied fruits and soft dates to overcome stickiness; limiting the season for canning desert grapefruit juice to months when the natural ratio of acid to sugar is such as to give an acceptable product; and use of drum driers and other idle equipment in distilleries for producing white potato flour.

This report gives the final results on three projects that have been completed. One is the production of feed yeast from citrus-cannery waste liquors; the second is the preparation of a canned juice of uniform quality from desert grapefruit throughout the season; and the third is a study of the role of enzymes in deterioration of eggs and egg products.

The research on extracting the new drug rutin from buckwheat plants, which was an offshoot of research on tobacco utilization, has resulted in the establishment of a new industry in less than 6 years. Six manufacturers of alfalfa meal have taken up the dehydrating of buckwheat plants, and 15 pharmaceutical firms are extracting rutin from buckwheat leaf meal or preparing it for medicinal use. When rutin comes into general professional use for treating diseases for which it has proved to be beneficial, about 50,000 acres will be required to produce buckwheat for rutin manufacture. The income to farmers from this acreage should total at least $2,000,000 per year. If the entire crop is converted to leaf meal, selling for $250 per ton, the dehydrators should receive more than $9,000,000, and the rutin produced should have a sales value of about $150,000,000. The cost of the research on rutin has been about $300,000 for salaries and expenses. Even if the entire cost of the Eastern Regional Laboratory and its equipment—about $2,000,000—is considered, the estimated return from this investment is very high. Similarly, the estimated returns from the research on penicillin, which made possible its commercial production in wartime, much more than justified the cost of the research and the entire cost of the Northern Regional Laboratory.

Not all of the Bureau's work can be expected to show such high returns for the money spent. Some research fails to attain the desired ends, but all of the Bureau's research is intended to yield results that are beneficial alike to agriculture and the industries that use agricultural materials and therefore to the entire Nation. The administrative officers of the Bureau believe that, in the long run, the money appropriated for research on the utilization of agricultural products is well spent and intelligently administered.

Some of the Bureau's work is discussed more fully in the 310 publications and articles issued and the 49 patents granted during the year.

COTTON-MARKET SURVEYS GUIDE RESEARCH

Economic and technological surveys made at the Southern Regional Research Laboratory during the past year point to the need for additional research on cotton textiles, as well as the direction this research should take, if cotton is to maintain its lead in competition with synthetic fibers and paper in tomorrow's markets. It is clear that cotton has lost its former price advantage over rayon, but the economic studies indicate that this advantage may be regained if the cost of production can be lowered.

A comprehensive study of the synthetic fibers that compete with cotton, made to determine the extent of their inroads into cotton's end-use markets, showed that consumption of these fibers is increasing rapidly as their quality is improved. It is evident that cotton will be able to maintain first place among the fibers consumed in this country only if more intensive research is devoted to improving the quality of cotton products.

To make the need for this type of research more widely known, a previous survey on trends in the consumption of fibers in the United States was brought up to date and published in mimeographed form (ACE—93 Revised—Preliminary Edition). Presenting data for the years 1892 to 1947, the report will serve as a primary source of statistics on fiber consumption and will be of major value to the Government in connection with basic studies of the national economy as well as to growers and processors of textile fibers.

Studies were also made during the past year on the amount of cotton used for minor products such as clotheslines and nonwoven (knitted, felted, cemented) fabrics. Cotton clotheslines were found to provide a market for 65,000 to 96,000 bales of cotton a year. Only about 40 percent of the 2½ to 3 million pounds of nonwoven fabrics produced during 1947 was cotton, whereas 60 percent was rayon. Both of these outlets for cotton can be enlarged through research.

WIDER USES SEEN FOR IMPROVED COTTON FABRICS

Cotton is still the best all-round textile fiber, having maintained its lead in the face of the recent remarkable improvements in rayon and other competing materials. Nevertheless, in the years immediately ahead this dominance can be assured only if unusual progress is made in preparing better textile products from cotton fibers. Even the properties in which cotton is at present admittedly superior, and certainly those in which it is less outstanding, must be improved if the markets for cotton are to be extended or maintained.

The Southern Regional Research Laboratory is exploring all possibilities for improving cotton textiles through chemical finishing, and has made notable progress in rot resistance and weather resistance, two of the principal qualities in which cotton needs improvement.

Rotproofing.—Commercial applications are developing in several fields for partially acetylated cotton which previous reports have described as highly resistant to mildewing and bacterial rot. Two manufacturers have decided to treat ready-made bags for water-softening systems, a use in which high resistance to rot without toxicity to animals is an essential. Technical assistance and advice given by the laboratory to one of these firms, treating such bags at the rate of 400 a day, resulted in simple, successful replenishing and repeated reuse of the acetylation mixture, thus avoiding a substantial item in the usual cost of recovering the chemicals. This installation is expected to demonstrate the possibilities of applying acetylation with relatively simple stock equipment for special purposes where the cost of the process will be far more than offset by enhanced value resulting from the increased life of the fabric after treatment.

Reports from outside sources continue to confirm the Bureau's findings that partially acetylated cotton products have unusual resistance to damage by micro-organisms in various situations.

Numerous tests have shown the possibility of protecting cotton under such severe conditions as burial in soil or exposure on the ground for periods ranging from 6 months to 2 years.

The strength of partially acetylated cotton twine used for fish nets, now being tested in sea water, is holding up remarkably well in comparison with that of twine subjected to other treatments. Untreated twine failed within 1 month in service.

Weatherproofing.—Although cotton already has wide use in outdoor fabrics—awnings, tents, tarpaulins, shade cloth, seed-bed covers, etc.—its consumption for these end products could be increased by chemical treatments that would extend their service life. Very satisfactory progress was made during the past year in developing treatments to make cotton fabrics withstand exposure to sunlight and other elements of weather without damage to their textile qualities. Special attention was given to the preparation and evaluation of treatments for shade cloths and ducks in different kinds of service. The progress made in this work, which resulted largely from the cooperation of farmers and industrial producers with the laboratory, is a good example of the benefits to be derived from cooperation between research groups and those interested in the end uses of the improved cotton.

Cotton goes into about 60 million yards of tobacco shade cloth annually. If a more durable type of cotton cloth were available for this use, undoubtedly larger acreages of tobacco would be covered by cotton. As a result of interest by Florida tobacco growers in an economical weather-resistant treatment for cotton shade cloth, a formulation was selected which appears practical from the finishing standpoint and in respect to cost. It involves the precipitation in the fiber of lead chromate, an inorganic yellow pigment, which screens out the sun's rays responsible for deterioration. Several full-width (50-inch) 50-yard pieces of the cloth treated with this pigment at the Southern Laboratory were sent to Florida for actual trial in the fields. Subsequently a private firm in Florida, using laundry equipment and with supervision by one of the laboratory's technicians, treated several thousand square yards—sufficient to cover several acres of tobacco plants. Arrangements were made to get samples of the used cloth at the end of the season for evaluation of performance.

In efforts to protect cotton army duck from damage by sunlight, light-screening, clear resins were applied alone and in combination with light-screening-pigment finishes. The combined resin-pigment treatment definitely improved weather resistance. Samples of duck treated with urea-formaldehyde resin in combination with lead chromate showed less than 5 percent loss of strength after a year's exposure to weather, compared to a loss of about 50 percent by untreated fabrics. In the treatment of colored-stripe awning ducks only clear, light-absorbing resins were tried since treatment with pigments would obviously mar the color effect. The effectiveness of urea-formaldehyde resin in this type of treatment was clearly established.

Other chemical treatments.—A new type of cotton fabric—"aminized" cotton—is produced by a chemical finishing process recently developed at the Southern Regional Laboratory. 2-Aminoethylsulfuric acid is allowed to react with the cellulose of the fabric in a strongly alkaline solution, whereby amino groups are chemically combined with the cellulose. This procedure is simpler and less expensive

than others proposed for the same purpose. The aminized cotton, unlike ordinary cotton, has the ability to take acid wool dyes readily. Moreover, the introduction of amino groups permits the addition of metallic elements to give rot resistance or organic groups to give water repellency or other new qualities to cotton for specific uses. Patents covering the development of such modified cellulose in cotton fabrics have been applied for.

Optimum conditions were established for the introduction of the carboxymethyl group ($-CH_2COOH$) into the cellulose molecule in both yarns and cloth. It was found that this treatment increases the rapidity with which cotton cloth takes up water, which suggests its possible use on toweling and similar materials. Some experiments were made to learn the properties of the metal salts of carboxymethylated cotton goods.

COTTON-PROCESSING RESEARCH MOVES FORWARD

Improvements in the uniformity of cotton yarn, cords, and fabrics would raise their quality and add to the serviceability of the finished product. Also, greater uniformity at the various stages in the manufacturing process, by increasing production efficiency and reducing costs, would place cotton products in a stronger competitive position. In the experimental cotton mill at the Southern Regional Research Laboratory attention is being given to the opportunities for getting more uniform cotton products at every stage of production—from the time the matted lint enters the opening machine through all the steps of processing into yarns and fabrics. There have been several significant developments in this work during the year.

Processing studies have been conducted on several varieties of cotton of different staple lengths with the idea of improving the uniformity and the appearance of the resultant cotton yarns and other products. These studies show that substantial improvements in uniformity can be secured by a better proportionment of drafts on the rear and front drafting zones of the long-draft roving frame. A new draft guide has been developed for a high-draft system using dual-zone drafting. This guide substantially improved the uniformity of the roving and reduced the variation in the single yarns by about 50 percent in strength and 40 percent in yarn number. Other processing studies have shown that increases in draft on drawing (4 to 8.02) and roving frames (8.02 to 16.13) do not affect materially the strength or grade of a coarse yarn. On the other hand, increases in spinning draft (12.6–25.2) result in significantly lower skein strength and have little or no effect on the grade of the yarn.

The growing substitution of machine picking for hand picking of cotton has increased the importance of more efficient equipment for cleaning ginned cotton. With a view to establishing some fundamental principles of cleaning, studies on the characteristic adherence of cotton fibers to trash and the electrostatic potential of the many types of trash particles were begun. But since a long time must elapse before sufficient knowledge to design complete cleaning equipment can result from such studies, work has gone forward on improving an opener, designed and constructed at the Southern Regional Research Laboratory, which opens and fluffs up matted lumps of cotton into small,

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soft tufts to prepare them for subsequent cleaning. It handles a bale of lint in less than an hour. The limited trials made so far show that the increased fluffiness is achieved without damage to the fibers. The design is flexible enough for use in getting information of research interest on each lot of cotton. Although trash removal is only incidental to the real function of the machine—the separation of matted cotton into loose fibers—several modifications are contemplated which should increase its cleaning action.

PROGRESS MADE IN RESEARCH ON COTTON TIRE CORD

Much practical knowledge was gained during the year in research at the Southern Regional Laboratory on cotton tire cord, which for many years has been the most important single end use of cotton. In this research valuable technical assistance was given by the tire-manufacturing industry.

The ply-building machine, mentioned in previous reports, is now producing plies for experimental tires for use in current studies on the fatigue life of cotton tire cord and on the effect of the number of yarns in the ply and the number of plies in the cord. The machine was moved to the plant of a cooperating tire-manufacturing company where the experimental cords can be made into plies at the source of the rubber stock, thus facilitating impregnation and coating of the cord with skim stock and application of the tread stock to the prepared carcass. Several tire companies are interested in duplicating this machine which provides a rapid and inexpensive method of making test tires from small lots of experimental tire cord.

A number of additional tire manufacturers requested and received the designs of the laboratory's constant-tension tire-cord stretcher described in last year's report. Several reported that they are contemplating the construction of similar machines.

One typical laboratory investigation during the year was concerned with determining the flex life of cotton tire cords. The experiments showed that cord failure is the result of total flexing action and that the tendency toward fatigue is not overcome during intervals of relaxation after flexing. Other laboratory experiments indicated that the evaluation of lubricated tires should be at the operating temperature of the tire. Further studies on the chemical degradation of the cellulose in cotton tire cord at various stages of manufacture when heated at different temperatures, as indicated by cuprammonium fluidity measurements and strength changes, confirmed the previous conclusion that encasement in rubber offers some protection against degradation.

Two of the four road tests started in April 1946 on truck tires made with three different types of cotton cord and one of rayon in distinct classes of service are still in progress. Two tires on the trailer wheels of the bread truck have run 225,000 miles, with about 25,000 miles of this total on the second recap. Four tires on the rear tractor wheels of the furniture van have run 63,305 miles and were recapped at this mileage for the second time.

Considerable improvement was made in laboratory equipment for testing tire cords. Work along this line included remodeling of a multiple-stand flexing machine to make it complete eight tests in the time required by the original model for two tests. The remodeling

machine requires only one technician's time for full operation. Another tester, for determining rate of fatigue under sharp and rapid flexing, was redesigned and rebuilt to test four specimens simultaneously. Effort was also directed toward adapting strain gages to more rapid measurement of some of the elastic properties of tire cords.

NEW TESTS AID DEVELOPMENT OF USES FOR COTTON

A good test for the appraisal of water absorbency in cotton fabrics has long been needed. Such a test for light-weight fabrics was developed during the past year at the Southern Regional Research Laboratory. It is made with simple equipment and consists merely of determining the time required for a disk of the fabric to absorb a measured volume of water from the surface of a porous plate. This procedure gives a better evaluation of absorbency than either of two others in current use and has the advantage of speed. A firm engaged in chemical finishing of cotton, which cooperated in this development, has successfully applied the technique also to heavy-weight, absorbent-type fabrics, including towelling. The new test should be useful in evaluating the absorbency of partially processed fabric as well as of finished goods.

A recent contribution to Textile Research Journal (February 1948) from the Southern Regional Laboratory described a technique known as the orifice test for evaluating cotton yarns for swelling-type water-resistant fabrics. In this test different samples of cotton in the form of yarn from, or intended for, woven fabrics are subjected to water under pressure after a small skein of the yarn, or bundle of parallel strands, having a definite previously calculated and adjusted weight per unit length is folded over a wire and drawn into an orifice of standard size. The relative ability of the wet cotton to close the orifice is determined by weighing the quantity of water forced through the orifice in definite periods of time after seepage is first observed.

A new method developed for determining the methoxyl group (CH_3O) in compounds of sugars, starch, or cellulose should aid in research on the break-down of cellulose by chemical agents. It is already being used in investigations on the structure of cellulose and the possibility of modifying cotton by introducing alcohol radicals into its molecules.

Conversion of cellulose to sugars (hydrolysis) by treatment with strong mineral acid has been used as a new tool for determining the fine structure of the cotton fiber since their different speeds of dissolution serve to differentiate between the amorphous and crystalline cellulose in the fiber. Correlation has been found between the results by this method and the ability of a bundle of the fibers to become more compact when wetted, as measured by a modification of the orifice test.

NEW KNOWLEDGE OF COTTONSEED PIGMENTS AIDS PROCESSING

Cottonseed, no less than cotton fiber, is facing a highly competitive situation. In recent years soybeans have not only been produced in larger quantities, but have been found more adaptable to solvent methods of processing. Research to improve extraction methods or to develop new methods is therefore clearly needed if cottonseed is to hold or expand its markets.

Over a long period little progress was made in solving the problems of recovering all of the oil from cottonseed kernels and meal. The two mechanical methods in general commercial use—hydraulic and screw pressing—are capable of giving high-grade oil and meal, but hydraulic pressing leaves 5 to 7 percent of the oil in the meal, and screw pressing leaves about 4 percent. Recovery of this oil would increase the annual cottonseed-oil production by over 100 million pounds. Moreover, the elimination of precooking, which is required in both methods, would result in improved meal containing unaltered protein adaptable for new industrial uses. Lately, solvent-extraction methods for processing cottonseed have been used commercially in the United States. Solvent extraction removes nearly all of the oil from the meal. It does not require cooking of the seed, but some preheating of meats and final toasting are usually necessary if the meal is used for feed. But so far none of these methods has reduced pigmentation of oil and meal. Therefore, in its cottonseed-processing research the Southern Regional Laboratory has sought a greater understanding of the complex cottonseed-pigment system. During the past year it acquired additional data on the nature and occurrence of cottonseed pigments which are expected to have direct application in the improvement of both mechanical and solvent methods of oil recovery.

Previous mill-scale research on the color of cottonseed oils obtained by hydraulic- and screw-pressing methods under different conditions of processing had shown that hydraulic-pressed and bleached oils from wet-cooked seed had a lower initial color and darkened more slowly during storage than screw-pressed oils from dry-cooked cottonseed. In both laboratory and mill-scale experiments, the effect on final pigment content of moisture added during precooking of the seed has been determined. The laboratory results showed that changes in the composition of pigments during cooking of the seed with added moisture result from reactions inside rather than outside the pigment glands. The mill-scale experiments showed that the addition of relatively large amounts of water together with high temperatures during cooking gave oils of the lowest color after bleaching and that in this way screw-pressed oils of prime color can be obtained.

Experiments by two cooperating universities indicated that the meal from dry-cooked, screw-pressed seed has higher nutritional value than that from wet-cooked, screw-pressed seed, but that the crude oil from the dry-cooked seed is initially toxic, while that from the wet-cooked seed is nontoxic. One oil mill is using dry cooking with the screw-press method to obtain the highly nutritious meal. The toxic factor in the oil is removed by standard refining procedures.

In experiments on the recovery of cottonseed oil by solvent extraction, about 14,550 pounds of cottonseed meats were extracted with commercial hexane in five runs of the continuous equipment in the pilot plant. Useful engineering data were obtained, and much was learned about the conversion of kernels to flakes that can be extracted without disintegration. Most of the meals obtained were of sufficiently low-fat content for use in protein extraction and fractionation studies.

In the year's research on cottonseed pigments an analytical method was developed for determining gossypol in seed, oil, or meal. This method has made it possible to determine the effect of the various methods of processing cottonseed on the gossypol content of the prod-

ucts. It combines three methods developed in previous work for the quantitative extraction of gossypol and includes a spectrophotometric test based upon the reaction product of antimony trichloride with gossypol.

SPOILAGE OF MOIST COTTONSEED IN STORAGE REDUCED BY CHEMICAL TREATMENTS

Spoilage of moist cottonseed during storage is a serious problem to the cottonseed-oil industry, except where the climate is excessively dry. In some years losses amount to millions of dollars. The increased respiration of the seed causes heat to accumulate within seed piles in the storage bin, and this results in deterioration of the seeds. Better methods than those used at present are greatly needed to meet this situation. For several years the Southern Regional Research Laboratory has cooperated with cottonseed-oil mills in storage experiments to solve this problem, and last year considerable advance was made in the use of a chemical treatment applied by spraying the seeds before they were stored.

Over 300 chemicals have now been tested in the laboratory. The 45 of those shown to be effective include simple esters, acid-alcohol combinations, compounds related to sulfa drugs, and some simple organic acids. Several of the most promising chemicals have been applied in actual mill-storage tests. Although added in very small amounts (less than 0.4 percent on the dry weight of the seed) these chemicals checked deterioration appreciably when used either alone or in mixtures. The success of the chemical treatment is equal to that of the aeration method commonly used, in which the stored seeds are cooled periodically by circulating air through the bins. Progressive experiments in the laboratory have indicated definite steps that must be taken to completely prevent deterioration on a commercial scale. The interest of oilseed processors in developing the method has been demonstrated by numerous invitations to carry out work in their mills. The chemical treatment has also been applied successfully to moist flaxseed and rice and should be applicable to grains in general.

From other laboratory experiments information was obtained on conditions under which research samples of cottonseed can be stored with a minimum of change in chemical composition. It was found that seed containing less than 8 percent moisture can be stored indefinitely at 0° F. Change in the characteristic light absorption of certain constituents of the extracted oil was established as a new index of deterioration.

PEANUT PROTEIN SHOWS PROMISE OF NEW INDUSTRIAL USE

Last year the Southern Regional Laboratory reported a fast-growing interest by industry in the use of solvent-extracted peanut protein in several special commercial fields. One of these specialized uses is as the adhesive in mineral coatings for paper and cardboard to be used in printing halftone pictures. The principal function of the adhesive in a paper-coating formulation is to bind the layer of mineral matter so firmly to the raw stock that it will not be removed by the pull of ink during printing or similar operations. Over 250 formulations using several types of mineral and different mineral-protein ratios at various pH values and with different dispersing agents were

tested in cooperation with industrial firms. These tests, besides confirming previously reported successful results, supplied information for optimum utilization of the protein.

Limited industrial production of peanut protein suitable for spinning into fiber is already under way. One firm which has been producing such protein on a pilot-plant scale recently announced its intention of going into full-scale production. Peanut protein for use in fiber and adhesives must have a high degree of solubility or dispersion in solutions of relatively low pH values. Only the protein prepared from solvent-extracted meal meets these conditions. The method of separating the protein from the meal was simplified, and the yield of protein was increased.

Most of the difficulties of pilot-plant solvent extraction of peanuts were overcome. A simple process for separating the skins from the peanuts with an air blast after heating sufficiently to rupture them greatly reduced the objectionable color imparted by the skins to the meal and protein. Flakes sufficiently tough and stable for successful extraction were produced on a laboratory scale by a treatment combining moisture with heat applied before flaking.

SWEETPOTATO-UTILIZATION PROCESSES IMPROVED

Progress was made at the Southern Regional Research Laboratory in further development of processes and equipment for the recovery of feed protein from waste waters previously thrown away in the manufacture of sweetpotato starch. In particular, the rapidity and efficiency of dewatering the crude protein concentrate by filtration and combining it with the byproduct pulp were much improved.

In four pilot-plant runs about 22,000 pounds of sweetpotatoes were processed for recovery of starch, protein concentrate, and byproduct pulp. About 800 pounds of straight-run byproduct pulp, containing 1 to 2 percent crude protein, and 900 pounds of enriched pulp, containing 11.5 percent protein, were prepared for continuation of preliminary feeding trials at the Florida Agricultural Experiment Station. These trials, in which the protein-enriched pulp and the plain pulp plus cottonseed meal are compared as rations for both calves and chickens, have progressed satisfactorily during the year.

Acquisition of new centrifugal equipment has made it possible to demonstrate on a pilot-plant scale the efficacy of an improved and simplified process for extracting and refining sweetpotato starch continuously. The conventional batch-type centrifugal machine was successfully replaced with an improved continuous solid-bowl centrifuge recently made available commercially. The continuous centrifuge effects material reduction in labor requirement and enhanced efficiency of processing.

NEW VARIETIES OF SWEETPOTATO EVALUATED FOR INDUSTRIAL USE

Recognizing the need for new strains of starch-type sweetpotatoes better adapted to large-scale field production, the Southern Regional Research Laboratory aided several research groups in the evaluation of new varieties for industrial use. It cooperated with the Mississippi Agricultural Experiment Station in collecting and preparing samples from 1947 sweetpotato variety trials for studies of their starch con-

tent. Samples of 25 new seedling sweetpotatoes of both starch and table types, furnished by the Louisiana Agricultural Experiment Station, were examined for chemical composition and processing quality. These studies confirmed earlier observations that the starch possibilities were better with the white-fleshed varieties, which are characterized by higher total solids and starch content, lower sugars, smaller increase in sugars at the expense of starch after digging, and better processing quality than yellow-fleshed varieties or crosses of white with yellow varieties.

The purified starches from 22 domestic varieties of sweetpotato have been analyzed for amylose, the more useful of the two starch fractions for films, fibers, and plastics. The group, composed of established and new varieties of both starch and table types, exhibited considerable variation in superficial physical characteristics. The apparent amylose content of the nondefatted starches ranged from 17.5 to 21.7 percent with an average of 20.3 percent, but in 16 of the 22 varieties the value fell in the narrow range of 20.0 to 21.5 percent. The amylose content of cornstarch is about 25 percent. Although the results do not encourage the hope of discovering a trend toward high amylose content in any existing variety of sweetpotato, the quest is being continued in view of higher industrial value of starch composed chiefly of amylose, having straight-chain molecules like those of cellulose, rather than amylopectin, having branched molecules like those of pectin.

NEW WORK ON SOUTHERN FARM CROPS STARTED UNDER RESEARCH AND MARKETING ACT

An expanded program of utilization research on cotton, cottonseed, peanuts, and rice, supported by funds appropriated under the Research and Marketing Act, was begun at the Southern Regional Research Laboratory with the acquisition of technical personnel and specialized equipment. Two broad projects on cotton are concerned with fundamental characteristics of the fiber as a means of developing entirely new uses and with development of new and improved cotton products through chemical processing and mechanical treatment. Research on cottonseed is concerned with determining properties of, and finding uses for, pigment glands and gland-free meal obtained by a fractionation technique originated by the Southern Laboratory. The object of new research on peanuts is to make an improved peanut butter. As soon as installation of equipment for the manufacture of peanut butter is completed, studies will be undertaken to determine the effect of individual processing variables on the quality of the finished product. Rice-bran oil has been recovered by solvent extraction, and preliminary studies of composition and quality have been completed. Results of the work under Research and Marketing Projects are briefly reported below.

SWELLING OF COTTON FIBERS BY WATER MEASURED MICROSCOPICALLY

The swelling by water of individual cotton fibers was studied microscopically to obtain basic data applicable in the development of improved water-resistant fabrics. Samples of four varieties of commercial cottons were separated into thick-walled and thin-walled fractions by means of the differential dyeing technique, and photographs

were made of cross sectional areas in both the wet and dry state. Because of variability, 500 fibers from each sample were measured. Wetting increased the cross-sectional area by 26 to 33 percent for the thick-walled fibers and 21 to 31 percent for the thin-walled fibers. These results somewhat lessen the hope of finding a cotton having unusually high swelling capacity. The relatively high physical stability of cotton was shown by the results of similar tests on raw flax and rayon. Both of these fibers increased in cross-sectional area by approximately 65 percent when wetted with water.

PROCESS FOR FRACTIONATION OF COTTONSEED MEATS ADVANCED TO PILOT-PLANT STAGE

The process-development research on fractionation of cottonseed was carried to the pilot-plant stage. In chemical engineering studies of unit operations of the process about 1,000 pounds of meal, essentially free of glands, oil, and hulls, and several pounds of pigment glands were produced for continued research on evaluation of nutritional, pharmacological, and chemical properties. The key step in the fractionation process is the disintegration of meats by a technique which detaches meal tissue from the glands without rupture of the gland walls. The most promising method tried depends on fluid friction to accomplish disintegration. In practice, flaked meats or partially defatted meal is placed in a solvent that does not affect the gland walls—for example, commercial hexane—and the resulting slurry is stirred by an impeller revolving at high speed. The variables of the disintegration step are being studied to determine the optimum conditions of operation. It was found that the moisture content of the prepared meats was an important factor; that flaked meats produced a better end product than disintegrated meal; and that variation in density of solvent had little effect. Separation of disintegrated flakes into a meal fraction and a pigment-gland fraction is another critical unit operation. Two ways of carrying out this step have been tested.

The 1946 annual report described the flotation method, which depends on the fact that pigment glands have a lower specific gravity than any other portion of the meats. Theoretically, if disintegrated meats were suspended with mixed solvent having a specific gravity intermediate between that of pigment glands and meal tissue, the gland portion would rise, and a separation would be made. In practice, difficulty was encountered in obtaining sufficiently pure fractions. Although the average specific gravity of the meal particles was greater than the average specific gravity of the glands, the difference was not always enough to permit quantitative separation. This was due in part to adhering meal tissue, which raised the apparent density of the glands, and in part to imbedded glands, which lowered the apparent density of the meal. It was found, too, that the actual density of the glands was raised by absorption of solvent, which limited the time during which separation by flotation could take place.

A second method of fractionation, based on the differential settling of glands and meal, was investigated during the past year. In this method the meats are disintegrated to fine-particle size in a low-specific-gravity solvent such as commercial hexane, and a partial separation is made by decanting the meal particles suspended in the solvent after the gland fraction together with some meal has settled to the bottom. This latter fraction can be further purified by the

flotation process. Centrifugal separation of meal, filtration, and removal of solvent are other unit operations being investigated in order to develop the principle of fractionation into a commercially feasible process.

PHYSIOLOGICAL EFFECTS OF COTTONSEED PIGMENTS DETERMINED

New knowledge concerning pigment glands and gland-free cottonseed meal was acquired in research carried out in cooperation with other laboratories. The acute toxicity of gossypol and cottonseed-pigment glands was determined for rats, mice, rabbits, and guinea pigs. It was found that ingestion of pigment glands in relatively large doses produced death in all these animals. Rats were the least sensitive, and rabbits and guinea pigs the most sensitive. The toxicity of cottonseed-pigment glands was attributed to some component or components other than, or in addition to, gossypol and gossypurpurin.

The nutritive value of cottonseed meals for chicks as affected by methods of processing and content of pigment glands was further investigated by cooperating federal and state agricultural research groups. In one of these investigations experimental and control diets were fed to parallel lots of 20 to 30 chicks for a period of 6 weeks. The basal diet contained 13 percent of screw-pressed soybean meal as the source of vegetable protein. In one experimental diet pigment glands were added to the basal diet; in another gossypol was added; and in all the others different cottonseed meals were substituted for the soybean meal. Gland-free cottonseed meal, uncooked diethyl ether-extracted cottonseed, and hydraulic-pressed cottonseed meal supported excellent growth, whereas markedly inferior growth resulted when the experimental diets contained uncooked hexane-extracted cottonseed or pigment glands. Since the pigment glands were present intact in the uncooked hexane-extracted meal, the physiologically deleterious factors were assumed to be segregated in the pigment glands. The addition to the basal diet of pure gossypol produced relatively little retardation in the growth of chicks, and a low correlation was found between the nutritional value of the different meals and their contents of gossypol and gossypurpurin.

Another cooperating group investigating the pharmacological properties of gossypol found that it has an appetite-depressant effect when fed to rats. Parallel experiments are being carried out with whole pigment glands as well as with gossypurpurin and other gland components. Plans have been made to extend the investigation of the appetite-depressant effect of gossypol when ingested by other animals and by human beings.

OIL RECOVERED FROM RICE-MILLING BYPRODUCT

All the food possibilities of rice have not yet been explored and developed, although rice is one of the most ancient and widely used foods in the world. One potential but undeveloped product is salad or cooking oil from the natural coating and germ removed from rice in milling. It is estimated that as much as 20 million pounds of oil might be recovered annually from the "bran" resulting from the United States rice crop.

The Southern Regional Laboratory has started work on the charac-

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terization and processing quality of oil obtained from rice bran by different methods. Four lots of oil of different histories—two extracted with commercial hexane in the Laboratory and two furnished by industrial cooperators—were refined, bleached, and deodorized. Each crude oil yielded a finished edible oil of acceptable grade, but improvement in methods is needed to reduce refining losses and to remove more of the greenish color attributed to the presence of chlorophyll. The stability or keeping quality of these oils varied considerably, although even the least stable compared favorably with a good grade of cottonseed or peanut oil. Evidence obtained by two methods indicated that natural antioxidants other than tocopherols may be responsible for the high stability values observed.

TEXTILE FIBERS MADE FROM CORN PROTEIN

In 1946 the Northern Regional Research Laboratory announced a laboratory process for the preparation of textile fibers from the corn protein zein. These fibers were shown to be strong and resilent, their dry strength being equal to that of wool. The wet strength of the fiber, which was about half of the dry strength, exceeded that of any synthetic protein fiber available commercially. The interest aroused by samples of cloth containing zein fibers indicated that the fibers should receive a ready acceptance by the trade. Mixtures of nylon, rayon, or cotton yarn with zein yarn gave interesting cloth combinations which had good drape and handle.

In order to expedite the production of zein fibers on a commercial scale, information on this process was disclosed to interested companies. One company immediately undertook laboratory studies and later made experiments for more than a year in a pilot plant having a rated capacity of 100 pounds per day in order to obtain pertinent data on the manufacture of the fiber. In February 1948 this company acquired a plant that was formerly used for the manufacture of textile fibers from casein, and in May 1948 it began to produce textile fibers from plant proteins. The first fibers produced were made from the corn protein zein. The rated capacity of the plant unit for zein fiber is said to be 2,500,000 pounds per year.

NEW PROCESS DEVELOPED FOR MAKING PAPER FROM STRAW

Wheat straw has been a recognized source of pulp for the manufacture of paper and board for more than a century. Although paper was made from straw in the early industrial life of the United States, chemical wood pulp for paper manufacture, introduced about 1860, gradually displaced straw except for making corrugating strawboard, for which straw is better suited than wood. In this country 25 mills, consuming 800,000 tons of wheat straw yearly, produce half a million tons of corrugating board.

Straw is used in Europe and other countries not only for making corrugating strawboard but also for making fine and specialty papers. Holland, Belgium, Germany, Italy, and South American Republics—especially the Argentine—produced such fine papers from straw before the war. England, during the war, had to turn to the use of straw, and considerable interest is now being shown there in building new pulp mills adapted to straw.

After several years' study, the Northern Regional Research Laboratory has announced a new alkaline sulfite process for producing fine paper pulps from straw in bleached yields of 50 to 52 percent and at lower chemical costs. These yields are from 8 to 10 percent higher than any obtained previously. It is recommended that this pulp be used as a blend, perhaps up to 40 percent, with certain wood pulps to produce improved magazine, book, litho, offset, and other fine and specialty papers. It is not intended that fine papers be made from straw alone. Mill trials of the process have already been undertaken.

The reason that straw has not been used for making fine papers in this country is to a considerable extent an economic one. It is more expensive to collect, package, and transport straw than pulpwood. Through the efforts of the Northern Regional Laboratory, the strawboard and farm-equipment industries are working cooperatively to solve this economic problem. During the past year a wide educational program on the possibility of increasing farm income by sale of straw to industry was conducted jointly by the industries and the United States Department of Agriculture.

The implication of these developments is clear when it is considered that 95 million tons of wheat straw was grown in this country in 1947, of which about 40 million tons was burned or otherwise wasted. The requirement for paper and chemical cellulose pulps during this year was 22 million tons. Theoretically, the wasted straw could have produced about two-thirds of this requirement. At the present time a pulp shortage exists and pulpwood is being used faster than it is being grown.

MOLD AMYLASE FROM SUBMERGED CULTURE AIDS ALCOHOL PRODUCTION

Industrial acceptance has already been accorded the method developed by the Northern Regional Research Laboratory for preparing the starch-converting enzyme amylase by submerged culture of a mold for use in the production of alcohol, despite the fact that additional work is still in progress. It has already been demonstrated that the fungal amylase can be used to replace malt completely with no decrease in the yield of alcohol, and experiments are in progress to ascertain whether an increase in the yield of alcohol from grain may not be possible through the use of this starch-hydrolyzing liquor. One large distiller has investigated this process on a semiplant scale; another is preparing to test the process on an industrial scale; and several others are studying the process on a pilot-plant scale.

In the production of alcohol by the yeast fermentation of grain or other starchy material, it is necessary first to convert the starch into sugar. This is easily accomplished by the use of barley malt, which contains starch-converting amylases, but malt is rather expensive. As the result of a survey of more than 350 fungi, a few molds were found to be capable of producing considerable starch-hydrolyzing enzymes when cultured under submerged conditions. In laboratory studies, culture liquor from one strain, *Aspergillus niger* NRRL 337, could replace malt completely when it was added to corn mashes in the proportion of 10 to 20 percent of the final mash volume.

Amylolytic liquor was produced in the pilot plant in 250-gallon

batches by allowing *Aspergillus niger* to grow submerged in a medium consisting of thin stillage fortified with 1 percent of ground corn and 0.5 percent of calcium cabonate. Thin stillage is a byproduct obtained in the manufacture of alcohol from grain. The medium was agitated mechanically and aerated throughout the period of fermentation. After about 60 hours the liquor was ready for use in the conversion of 2,500 gallons of corn mash. Its efficiency was demonstrated by substituting it for malt in preparing the mash and comparing the yield of alcohol with that obtained when malt was used in the usual manner. Experiments also showed that thin stillage from a previous mold conversion could be used repeatedly for the production of amylase with no decrease in the yield of alcohol.

The pilot-plant experiments demonstrated that operation of the process on a large scale is practicable and feasible. Cost estimates indicated that enough amylolytic liquor to convert 1 bushel of grain could be produced for about 6 cents and that by using this mold preparation a distillery mashing 5,000 bushels of grain per day could reduce its cost of operation by as much as $480 per day.

RIBOFLAVIN PRODUCED CHEAPLY BY USE OF SPECIAL YEAST

Pilot-plant work was started at the Northern Regional Research Laboratory on the production of riboflavin by a very promising biological process which was worked out previously in the laboratory. Preliminary cost estimates indicate that the process will be commercially sound, and the results obtained to date are so encouraging that several industrial concerns are already investigating the method. Riboflavin, sometimes called vitamin B_2, is one of the growth-promoting vitamins, and commercial feed mixers use large quantities of it for the enrichment of prepared feeds, particularly those for hogs and poultry. In 1938 the price of the vitamin recovered from natural sources was $17.50 per gram, but in 1948 the price of chemically synthesized riboflavin was only 15 cents per gram. Riboflavin is now being produced commercially by both chemical synthesis and biological methods.

When a strain of the yeast *Ashbya gossypii* was first added to the culture collection at the Northern Region Research Laboratory, the microbiologists observed that, after a few days of incubation, it produced very pale yellow colonies having a greenish cast. The colonies became more highly colored in the periodic stock transfers to maintain viability, and more than a year later, a strain that formed bright orange-yellow colonies within a few days at room temperature was isolated. The orange-yellow pigment was found to be riboflavin. Since many micro-organisms synthesize vitamins in quantities exceeding their own growth requirements but few are capable of producing amounts sufficient to warrant industrial attention, the observation that *Ashbya gossypii* produced substantial quantities of riboflavin suggested that this micro-organism might be employed for industrial production of the vitamin.

The best medium developed in the laboratory for this micro-organism is a solution containing 2 percent of corn sugar, 0.75 percent of corn-steep-liquor solids, and 0.75 percent of animal-stick-liquor solids. The corn sugar serves as a source of carbon and energy for the yeast, and the other ingredients furnish necessary nitrogen, minerals, and

growth factors. Corn-steep liquor is a byproduct from the manufacture of cornstarch and corn sugar, and animal-stick liquor is a byproduct of the meat-packing industry. All of these materials are available in abundance at low cost. When a medium of the above composition is fermented by the yeast, the riboflavin formed remains dissolved in the residual liquor. Concentrations of 700 micrograms of riboflavin per milliliter of spent medium were obtained in laboratory fermentations. But, so far, the yields obtained in the pilot plant are not as high as those obtained in the laboratory. Optimum conditions have been established for sterilizing the medium, and experiments on the rate of aeration are being conducted. Because of some unavoidable variations in experimental conditions, the results are quite variable, but yields well in excess of 500 micrograms per milliliter are being obtained. Preliminary cost estimates, based on the assumption that a process can be developed to yield 500 micrograms of riboflavin per milliliter indicate that the vitamin can be produced for about 5 cents per gram.

SOYBEAN SALAD OIL IMPROVED

During the past 10 years, soybean production increased more than threefold. The demand for edible oil during World War II was primarily responsible for this increase. Soybean oil is much easier to process and refine than certain other vegetable oils, but its flavor stability is not considered to be as good as that of cottonseed or corn oil. This difference in flavor stability appears to be a primary factor in the trade, since crude soybean oil normally commands a lower price than crude cottonseed or corn oil. During the past year, differentials of between 4 and 9 cents a pound between crude soybean and corn oil have existed. The difference in flavor stability is primarily represented by the development of painty, fishy, and grassy flavors in soybean oil at low oxidation levels, whereas cottonseed oil gives rancid, burnt, and other flavors at comparatively high oxidation levels. During the existing world shortage of edible oils, large quantities of soybean oil must be used, but with increase in world supplies of other oils the demand for soybean oil may drop unless improved flavor stability is achieved.

Some progress has been made in correcting the flavor instability of soybean oil. The German procedure of adding citric acid during the deodorization of soybean salad oils, which was recently investigated at the Northern Regional Research Laboratory, has been tested by several large refiners and subsequently adopted for a portion of their production. This treatment improves initial quality and extends the shelf life of soybean salad oil threefold to fivefold.

However, the efficacy of another feature of the German procedure has not been substantiated. German processors considered that lecithin remaining in deodorized soybean oil was the cause of its flavor instability, and therefore instituted exhaustive water washing as a preliminary "degumming" step to remove the lecithin.

In laboratory experiments it was found that exhaustive degumming does not improve flavor characteristics of refined soybean oil and that lecithin itself can be added to the deodorizer with a distinctly beneficial effect. Citric acid, which the German processors added to "inactivate" remaining traces of lecithin, actually performs the function of a "metal

scavenger" by reacting with minute amounts of metallic impurities in the oil. In the absence of a metal scavenger, these metals speed up or catalyze the oxidation and flavor deterioration of the oil.

Other substances, such a mannitol, sorbitol, and phosphoric acid, were subsequently found to be highly effective as metal scavengers and are also being tested as soybean oil flavor stabilizers on a commercial scale. In fact lecithin itself contains phosphoric acid, and appears to exert its beneficial effect by functioning as a metal scavenger. Since all metal scavengers studied to date, excepting lecithin, do not have the property of being directly soluble in the oil, attempts to synthesize fat-soluble metal scavengers are now in progress.

Although much valuable information is being obtained by a study of the influence of processing methods upon flavor stability, it is becoming increasingly apparent that a more fundamental mode of attack is required. Knowledge of the chemical nature of the objectionable principle, of its precursors, and of the chemical reactions by which it arises will greatly aid in suggesting remedial measures. The new line of attack has been initiated this past year as a project under the Research and Marketing Act of 1946.

REFINING-LOSS TEST DEVELOPED FOR SOYBEAN OIL

In October 1947 the buying and selling of soybean oil on a refining-loss basis was started. While a laboratory method had been devised and adopted as official for determining refining loss, it had not been given the vigorous test of commercial trading. In general the results have been satisfactory, but with some degummed soybean oils the present official method is unsuccessful. Since an increasing amount of soybean oil is being degummed, this defect in the method is serious.

The Northern Regional Research Laboratory, in close cooperation with the refining committee of the American Oil Chemists' Society, sent out nine samples of oil for collaborative test by a new centrifugal refining method. This new method gave more closely agreeing results than did any of the older methods and good results on all degummed oils tested.

A multiple stirring machine was devised and built at the Laboratory to expedite the use of the method. In addition, numerous experiments were conducted to fix the conditions of this test, which is under consideration for official adoption.

Since the price of soybean oil varies by ¾ of 1 percent for each 1 percent of refining loss and since the present official method when used by different laboratories frequently shows differences of 2 or 3 percent in refining loss on the same oil sample, it is obvious that the development of a successful and accurate method for measuring refining loss is of great importance to the entire industry and is needed as a satisfactory base for trading between producers and users of this oil.

SURPLUS POTATOES PROCESSED FOR FOOD USES

The European Recovery Program created a demand for white (Irish) potato flour far in excess of this country's manufacturing capacity. To relieve this situation, a process was developed at the Eastern Regional Research Laboratory whereby the type of drum dryers and other idle equipment found in distilleries could be used

to produce potato flour. As a result, the production of white potato flour was increased more than threefold.

The recommended procedure is as follows: The potatoes are washed in a conventional-type potato washer to remove dirt and stones, immersed in a hot lye bath, and washed with high-pressure water jets to remove the skins. (Other methods for removing the skins can also be used.) After being conveyed over an inspection table where defective tubers are trimmed or removed, the potatoes are processed with low-pressure steam in batch cookers until soft, and are then passed through a hammer mill to produce a fluid paste having a thick, creamy consistency. The "creamed" potatoes are pumped to a double-drum dryer the drums of which rotate toward each other and downward. The dried product is conveyed to a hammer mill, ground, screened, and bagged. The success of the process depends largely on maintaining the temperature of the potatoes above 150° F. from the time they are cooked until they are dried and on the whipping action to which the cooked potatoes are subjected in the hammer mill.

Other methods for drying potatoes are being developed to produce a potato meal that can be used as a component of dehydrated soup mixtures.

SURVEY MADE OF POTATO UTILIZATION IN THE UNITED STATES

A survey was made of the current situation in potato utilization throughout the major producing areas of the United States during 1947. The total 1947 crop was estimated at 380 million bushels, about 95 million bushels below the record crop of 1946. Although the total crop was practically the same as the goal, poor distribution and perhaps further decrease in per capita consumption caused an apparent surplus. The Government found it necessary to purchase about 25 percent of Maine's crop. These potatoes went principally for export, starch production, school lunches, aid to institutions, and into storage outside the State. Slightly smaller than normal crops in the Red River Valley and Idaho resulted in shipment of perhaps a greater amount of Maine potatoes to the far West than ever before, although marketing difficulties outside of Maine's normal area of distribution were encountered.

It was estimated that Maine produced 44 million pounds of potato starch during the September 1946 to June 1947 campaign. An unprecedented water-power shortage due to low streams reduced the production to an even lower figure during 1947–48.

Processing of potatoes for food is decidedly on the increase in Maine. One company uses 2,300 bushels each 8-hour shift in its two plants for production of canned and frozen French-fried potatoes. Another plant produces a large quantity of frozen French-fried potatoes.

Potato processing in the Red River Valley sections of Minnesota and North Dakota is concerned principally with potato chips, potato flour, and dehydrated potatoes. A novel experiment in dehydration of potatoes for feed was tried in this region during the early part of 1947. Potatoes that had been allowed to lie in the fields during the winter and subjected to alternate freezing and thawing were satisfactorily preserved as dehydrated feed.

The potato areas of Idaho are well supplied with processing plants.

These plants include five for starch, five for flour, one for glucose sirup, and three for dehydrated potatoes. Idaho produces from one-third to one-half of the Nation's potato starch and possesses about 80 percent of its total normal capacity for producing potato flour.

Considerable potential capacity for producing potato starch is available in the Northwest. If dryers are installed, starch can be produced in two plants in Washington and one in California that were built for potato-sirup manufacture.

During the past 2 years the first large-scale operations in ensiling of cull and surplus potatoes have been carried out in San Joaquin County, Calif. If economically feasible, ensiling will provide a good method for converting potatoes into a stable feedstuff. The highest yields of potatoes in the country are obtained in this section, averaging about 460 bushels per acre.

Kern County, Calif., now ships over 20 million bushels of potatoes annually (about 65 percent to east of the Mississippi River), about half as much as Maine ships through ordinary marketing channels. Sun drying of potatoes for feedstuff or fermentation material has been practiced there for several years. In the southern California climate cracked potatoes dry to 10–12 percent moisture in 4 to 5 days.

Colorado has expanded its potato production to about 22 million bushels per year, of which about 10 percent are culls. The Colorado climate is apparently suitable for dehydrating naturally frozen potatoes, as was done in the Red River Valley. The Colorado Agricultural Experiment Station placed potatoes in crib storage in the fall of 1947 with the intention of dehydrating them in the following spring. The results of the experiment have not been published to date.

SURVEY MADE OF POTATO UTILIZATION IN EUROPE

Because of the Department's extensive program on the development of industrial uses for surplus potatoes, a survey was made of European practices, especially in countries whose agricultural economy is largely based on potatoes. Information was obtained in Germany on cheap methods for conversion of potatoes to stable forms for use as feed and food. In Switzerland information was obtained on portable units for cooking potatoes prior to ensiling them underground for feed. A compilation was made of information from Germany, Holland, and France on the techniques of manufacturing white potato starch and utilizing waste products from starch factories. The latter information was useful in the design of a pilot plant now under construction at the Eastern Regional Research Laboratory and is expected to aid in operating the plant for improving the yield and quality of white potato starch and for utilizing its byproducts.

POTENTIAL USES FOUND FOR ALLYL STARCH

Allyl starch as prepared at present has a number of actual and potential applications. If properly compounded with various plasticizers, pigments, and fillers, it makes a very hard and resistant coating for house and office furniture. Several industrial firms found a marked improvement in the gloss and durability of printing inks when allyl starch replaced some of the resins used in inks at present. One commercial firm finds use for 50,000 pounds of allyl starch annually for some undisclosed application.

The use of allyl starch can be extended considerably if its films can be made more resistant to weathering conditions and thus more suitable for outside coatings. This might be achieved through chemical modification of the allyl starch molecule; *e. g.*, by substituting the remaining hydroxyl group with an organic radical, or through copolymerization of allyl starch with other monomers, or through proper formulation of allyl starch with water-resistant ingredients. Final choice of the method will depend upon its relative cost.

It was reported previously that three commercial firms were making allyl starch on a pilot-plant scale. One of these firms has enlarged its scale of operation (still pilot plant) and sells the product in large experimental quantities or supplies small samples without cost to prospective users.

In cooperation with the Sugar Research Foundation, Inc., allyl sucrose, a product similar to allyl starch, was prepared from sucrose (ordinary beet or cane sugar). Several industrial firms are interested in this product. At present preparations are being made for producing allyl sucrose on a pilot-plant scale.

LACTOPRENE EV CAN REPLACE RUBBER FOR SPECIAL USES

At the Eastern Regional Research Laboratory the continued study of the preparation of Lactoprene EV, an acrylic rubber obtained by polymerizing a mixture of 95 percent ethyl acrylate and 5 percent chloroethyl vinyl ether has yielded improvements in the polymerization process. A latex of high solids content (55 percent) was produced on a small scale in a three-stage, continuous polymerization unit. By proper control of the catalyst concentration in the last stage, it was possible to obtain virtually complete conversion of the monomers into polymer, thus eliminating the stripping operation necessary to remove residual monomer. The continuous process, with its added advantages, produced Lactoprene which was equal in quality to that produced by a batch process.

Lactoprene EV is superior to natural rubber and to any of the other synthetic rubbers, excepting silicones, with respect to heat resistance and hence is of considerable interest to industry. In order to provide samples, as well as engineering data, a considerable quantity of Lactoprene EV was made batchwise in a pilot plant at the Government laboratories operated by the University of Akron as agent for the Office of Rubber Reserve of the Reconstruction Finance Corporation. Approximately 200 requests for samples soon exhausted this supply, as well as additional quantities prepared at the Eastern Regional Laboratory.

Within recent months one of the large rubber companies, which already had marketed an acrylic rubber of its own, decided to undertake the industrial exploitation of Lactoprene EV. It has started pilot-plant studies and expects to offer large experimental samples soon.

VEGETABLE WASTES CONTAIN ESSENTIAL AMINO ACIDS

In order to produce more meat, dairy, and poultry products there is need for ever-increasing supplies of protein feedstuffs containing the 10 essential amino acids—arginine, histidine, lysine, leucine, isoleucine, valine, methionine, threonine, phenylalanine, and tryptophan.

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As a contribution toward this need, the Eastern Regional Research Laboratory in its work on the utilization of vegetable wastes has found that the trimmings and residues from market vegetables are excellent sources of proteins containing well-balanced mixtures of these essential amino acids.

Vegetable-leaf meals containing from 18 to 43 percent of crude protein were prepared on a pilot-plant scale from field and packing-house wastes of beets, carrots, peas, lima beans, rutabagas, rhubarb, broccoli, and spinach. Celery-leaf meal is already in commercial production in Florida, and lettuce meal in California.

Individual amino acids were determined by recently developed microbiological procedures. The analyses showed that the leaf proteins of the different vegetable species are very similar in their content of the 10 essential amino acids, the average percentages for the 8 leaf meals being: Arginine, 4.04; histidine, 1.51; lysine, 4.38; leucine, 6.60; isoleucine, 4.99; valine, 4.81; methionine, 0.54; threonine, 3.68; phenylalanine, 5.65; and tryptophan, 1.27. These values were calculated on the basis of crude protein and with the factor 6.25 for converting nitrogen to protein.

Concentration of many of the leaf proteins was accomplished by a bacterial fermentation procedure and also by formic acid extraction, followed by removal of carbohydrates by precipitation with alcohol. The purified proteins showed from 25 to 35 percent higher values for all of the amino acids except methionine, making it apparent that the crude-protein value for leaf meal, obtained by multiplying the percentage of nitrogen by the factor 6.25, is too high, because there is considerable nonprotein nitrogen in leaf meal.

The values for methionine in the purified proteins are from three to four times as high as those for methionine in the leaf meals, 1.54 percent being found in the protoplasts and 2.12 percent in the products obtained by extraction with formic acid. Since carbohydrates interfere with methionine recovery during hydrolysis of the proteins and since both methods of purification remove carbohydrate, it is almost certain that the percentage given for methionine in the leaf meals is too low.

The vegetable-leaf proteins compare favorably with other feed proteins in their content of essential amino acids, being about equal to linseed and soybean-oil meals in all amino acids except arginine and histidine. They are equal to meat-and-bone meal, except in arginine, and superior to corn-gluten meal in arginine and lysine contents. Fish meals are higher in the basic amino acids and methionine but lower in tryptophan. Preliminary feeding studies with regard to weight production in broiler chicks showed that the leaf meals can replace 5 percent of meat-and-bone meal and corn-gluten meal, but gave slightly inferior results when replacing 5 percent of fish meal or 15 percent of soybean-oil meal.

NICOTIANA RUSTICA STUDIED AS SOURCE OF NICOTINE

The shortage of nicotine in recent years and perhaps for some years to come again focuses attention on the possibility of growing *Nicotiana rustica* for its nicotine content. This type of tobacco is grown for smoking in Russia and India but not here. It has about twice the nicotine content of ordinary tobacco. Its cultural requirements and

behavior have been ably studied by another bureau of the Department. Although rustica can be grown in the tobacco regions of the eastern part of the country, it produces the most nicotine in parts of the West. Since the existing factories for nicotine recovery are in the East, the problem becomes: (1) Should the plant be dried and shipped to the East? or (2) Should factories be built in the growing areas for recovery of nicotine from the fresh plant?

Since these questions are fundamental to the whole rustica proposition and since there is practically no published information either on the handling of rustica or on the current methods of recovering nicotine from tobacco, the Eastern Regional Laboratory has undertaken to study the whole problem from the beginning as a project under the Research and Marketing Act.

Rustica leaves can be dried to 10 percent moisture without appreciable loss of nicotine provided the temperature of the drying air does not exceed 110° C. Nicotine loss is about 7 percent when this temperature is exceeded and the final moisture content is about 3 percent.

Finely cut rustica cannot be ensiled by the usual methods—straight fermentation, or addition of molasses, or use of mineral acids—without loss of nicotine. If bentonite is mixed with the material the loss of nicotine is only 1 or 2 percent.

Fresh rustica can be distilled at atmospheric pressure to get a distillate of 0.8 percent nicotine content and a recovery of 95 to 97 percent. Poorer results are obtained under vacuum. If, however, the plant material is partially dried in vacuum, then limed and distilled at atmospheric pressure, distillates of about 1.5 percent nicotine content can be obtained.

Juice pressed from fresh rustica has the same nicotine content as the original material. Four or five pressings, with water added in the last two, recover about 92 percent of the original nicotine.

Since extraction of nicotine from ground rustica, press juice, or distillate, by organic solvents would be a useful procedure, a study of the distribution coefficient of nicotine between water and such solvents is being made. Some of the 20 solvents studied thus far, notably chloroform and dichloroethylene, are more efficient than the kerosene now in use.

The ultimate aim is to develop complete processes for the preparation of commercial forms of nicotine—40-percent nicotine sulfate solution and 99-percent alkaloid—from either rustica or tobacco waste.

PHOTOCHEMICAL OXIDATION OF NICOTINE YIELDS NEW CHEMICAL

When a water solution of nicotine containing methylene blue is exposed to light, oxygen is absorbed. The reaction stops when each molecule of nicotine has taken up one molecule of oxygen. The product has been isolated and found to have a molecular formula equivalent to that of nicotine dioxide multiplied by 2, which is $(C_{10}H_{14}N_2O_2)_2$. It could properly be called a di(nicotine dioxide). The places of attachment of the four oxygen atoms have not been determined but are under investigation. The most effective wave length of light for bringing about the reaction is 6700 A., and the optimum pH is about 9.

While this work was in progress it was learned that another investigator had isolated from fermented cigar tobacco a compound that

seems to be identical with the one described. Some 30 to 40 percent of the nicotine in the leaf is converted to this compound during fermentation. By cooperative work between the laboratories involved it is hoped to establish the common denominator of these two diverse processes for producing the same substance and to discover the significance of both reactions. Furthermore, the new substance may become a yardstick for measuring the progress of fermentation in tobacco. Thus, although di(nicotine dioxide) itself may prove to be an unimportant compound, the photochemical reaction involved may have wide application in the modification of biologically active compounds and in the study of tobacco fermentation.

RUTIN-EXTRACTION PROCESS IMPROVED

Considerable improvement in the process for extracting rutin from either green or dried buckwheat has been made at the Eastern Regional Laboratory by using hot solvents, especially isopropyl alcohol, in the extraction. Isopropyl alcohol diluted with not more than 30 percent by volume of water can be used to extract dried buckwheat. Stronger isopropyl alcohol is recommended for use on the green plant. With a hot solvent the time of extraction is reduced to 10 minutes, and the process is more efficient than when cold solvents are used. This process is already in use in at least two factories that are producing rutin on a commercial scale.

Experiments conducted in cooperation with the Pennsylvania State College and the Bureau of Plant Industry, Soils, and Agricultural Engineering for a period of 3 years have indicated very plainly that the Tartary species of buckwheat (*Fagopyrum tataricum*) is superior to the Japanese species (*F. esculentum*) for the production of rutin. The Tartary species contains more rutin and yields considerably more of the gulcoside per acre than does the Japanese species. It also has the advantage that the date of harvest is not so critical as in the case of Japanese buckwheat, which should be harvested within a short period (about 2 weeks). The Tartary buckwheat, may be harvested during a period of at least 4 weeks. This permits safe postponement of harvesting when unfavorable weather conditions or other delaying circumstances occur. As a result of the experiments all of the buckwheat now being grown for rutin production is of the Tartary species.

Commercial manufacture of rutin has been expanding. Two new plants for the manufacture of this substance have been erected, and several new companies have begun the commercial preparation of the drug. A considerable quantity of rutin is being exported, particularly to Latin-American countries. Reports indicate that a number of European factories as well as at least one drug company in Australia are engaged in rutin manufacture.

Clinical applications of rutin continue to expand. With an adequate supply of the drug available to physicians, the use of rutin has been increasing and its application has been extended to other diseases. Perhaps the most spectacular development has been the discovery that rutin is able to protect dogs from the harmful effects of midlethal doses of X-irradiation. This suggests that rutin may be a valuable prophylactic against injury from atomic radiation. Rutin has continued to give beneficial results in cases of hemophilia, in which it lessens the severity and duration of hemorrhage, decreases pain, and

effects a general improvement in the condition of the patient. In cases of retinitis not complicated by diabetes rutin has proved effective in preventing retinal hemorrhage. Rutin has been used in glaucoma to correct capillary permeability resulting from the use of miotics in treating this disease. An important development is the internal use of rutin in cases of frostbite, in which it effectively prevents gangrene and the consequent loss of tissue. Rutin has been used with success in the treatment of Eales' disease of the eye, in which there are hemorrhages into the retina and vitreous humor.

The earlier ideas about dosage with rutin were based on the erroneous belief that rutin is rapidly excreted from the body, since statements in the literature indicated rapid excretion of the flavonol glucosides. It now appears that rutin should be given in large doses at the beginning of treatment and slowly tapered off to a maintenance dose, which can be expected to result in more rapid appearance of beneficial results.

The discovery that rutin is not rapidly excreted from the body but apparently is stored until a certain concentration is attained resulted from the use of a new analytical method developed at the Eastern Regional Laboratory for determining rutin in urine. The new technique, which requires a colorimeter for the final measurement, permits the removal of interfering nonrutin pigments that have heretofore vitiated the results and the detection of 0.01 mg. of rutin per milliliter of urine (about one-thousandth of 1 percent).

RESEARCH ON POTENTIAL TANNIN SOURCES CONTINUED

The increasing demand for more adequate supplies of domestic vegetable tannins has been emphasized recently by stock piling of tanning extracts for use in event of a national emergency, and by doubling of the price of quebracho extract—the most important imported tannin. Considerable progress has been made in the research work on the development of a domestic tannin supply which is being conducted at the Eastern Regional Research Laboratory in cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering. The principal emphasis is being placed upon canaigre roots and sumac leaves because such materials could be produced as tannin-bearing crops. Further work has also been conducted on scrub oak barks and nutshells as possible sources of tannin.

Canaigre roots.—Experimental plots of canaigre grown at State College, N. Mex., as a part of the cooperative program, and harvested in 1947 after 2 years of growth, produced about 10 tons of roots. These are being used for planting stock and for extraction studies. Thirty-two New Mexico canaigre strains, the progeny from which had been replanted successively for several years, showed, on a basis of harvests from 32 to 50 hills, average calculated yields ranging from 10 to 21 tons and averaging 16 tons of roots per acre per year.

Water-requirement tests including 5 different irrigation treatments replicated 4 times were inconclusive as regards root yields. Analyses of 32 root samples, however, showed that the tannin content was not materially affected by the various irrigation treatments, either in 1- or 2-year crops. On the moisture-free roots the tannin content, determined by extraction with 50-percent acetone, for the 1-year crop ranged from 26.0 to 28.2 percent and averaged 27.0 percent. For the

2-year crop it ranged from 25.8 to 26.8 percent and averaged 26.4 percent.

A study of the effect of different spacing of the plants on the tannin content of their roots showed no marked differences in tannin content of roots resulting from the various spacings tried. The results, however, indicated that the total yield of tannin per acre would differ with different spacings. The lower yields of roots per hill resulting from crowding of plants would be more than compensated by the larger number of hills per acre in the closer spacings.

To obtain quantities of canaigre roots needed for large-scale laboratory extraction studies, root and seed plantings totaling about 8 acres were made in 1947 with New Mexico planting stock. These plantings, which were located at the U.S.D.A. field station at Yuma, Ariz., and its branch at Bard, Calif., showed very satisfactory germination and plant growth.

In connection with the adaptation of successful laboratory extraction procedure to pilot-scale operation, comparative tests with different types of comminuting machines for preparing the roots showed that a cutter with knives that have a shearing and mixing action gives a product better adapted to efficient extraction than the other types of cutters tested. In comparing different methods of dewatering canaigre-root slurries, it was found that screening and pressing gives as effective dewatering as centrifuging.

Laboratory extractions of cut and dried canaigre roots showed that the characteristics of the extracted liquors vary to a marked degree. When first obtained from finely cut or sheared canaigre shreds by mixing with water, the liquors are usually turbid. They differ widely, however, in their response to settling. Some settle rapidly, giving practically clear liquors that can be concentrated without further clarification. In other cases there is no appreciable clearing after long settling.

An investigation of factors which might be responsible for the nonsettling property showed that either of two known conditions could produce this difficulty. One is improper drying of freshly shredded roots and the other is the use of immature roots. Current-growth roots harvested during the active growth period from February to May gave turbid nonsettling liquors, whereas roots from the plot harvested from July to September, during their dormant state, gave liquors that settled rapidly and yielded almost clear solutions. Examination of the material precipitated from turbid suspensions indicated that the turbidity was not entirely due to the presence of starch, but might be caused by a complex compound of protein and tannin, with starch acting as a protective colloid that hindered precipitation. It appears that this compound is present in young roots but disappears as roots mature. A similar substance responsible for persistent turbidity of liquors may result from improper drying of mature roots. These results emphasize the desirability of harvesting canaigre during July and August, which period has already been indicated as best as regards yield, tannin content, and purity of extract.

To obtain tanning extracts of high purity, it is necessary to remove the sugars that are extracted with tannin from canaigre roots. This has been accomplished in laboratory tests by fermentation with certain strains of Aerobacter. The liquors from which sugars were re-

moved by fermentation have been successfully used in the laboratory preparation of tanning extracts of high purity and high tannin content. In continued investigations of the Aerobacter fermentation of canaigre liquor 14 cultures were tested for their ability to produce 2,3-butanediol, acetoin, and ethanol when grown in canaigre liquors. Four of the better cultures were used in studying the fermentation of Arizona and New Mexico canaigre liquors, and comparable data were obtained on time of fermentation, percent of total sugar fermented, yields of 2,3-butanediol, acetoin, and ethanol, and purity of fermented liquors.

It was found that the rate of aeration during fermentation influences the yield of products as well as the rate of sugar destruction. The highest yield of products is reached when the sugars have been completely fermented. This point can be determined by measuring specific gravity and refractive index of the liquor. Added nutrient salts also affect the yield of products. Ammonium nitrate increases the amount of diol produced but slows down the fermentation rate markedly. Potassium acid phosphate plus ammonium nitrate decreases the fermentation rate and seems to have little, if any, effect on the diol yield.

A systematic study of the extraction of canaigre roots for the determination of tannin showed that concordant and maximum values for tannin were obtained with 25-percent acetone at temperatures ranging from 23° to 50° C. The results suggest the possible use of 25-percent acetone for the commercial extraction of tannin from canaigre roots, provided that such extraction is economically feasible.

An engineering adaptation of the laboratory-developed process for the water extraction of tannin from canaigre root was made for pilot-plant studies of extract preparation. The pilot plant, which has been set up and is ready for a series of tests, provides for the following operations: Comminution of shredded roots; countercurrent extraction of roots in a series of tanks having mechanical devices for mixing the root material with water or liquor at 40°–45° C.; separation of liquor from coarse solids by means of a vibrating screen and more complete dewatering of the solids in a continuous press; removal of fine solids from head liquor by settling and centrifuging; removal of sugars by fermentation; removal of insolubles by centrifuging; and concentration of the liquor by vacuum evaporation to a liquid tanning extract. Some experiments are in progress looking toward the recovery of volatile fermentation byproducts in the pilot-plant tests. Plans are being made under a Research and Marketing Act project for the collection of several tons of canaigre roots and the use of the pilot plant for processing them into tanning extracts.

Sumac leaves.—Further work, in cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering, has been done on the development of domestic sumac as a source of tannin. Several lots of sumac leaves and stems from *Rhus glabra* and *Rhus typhina* that had been dried in different ways were used in tanning 189 sheepskin skivers. When graded, the tanned skins showed differences in leather quality. The best leathers were produced with good air-dried leaves, and leathers of inferior quality were produced with leaves that had been damaged in various ways and with leaf stems.

Similar drying tests were conducted on 275 pounds of green leaves and about 40 pounds of stems collected in 1947 from *Rhus copallina*

plants grown at Beltsville, Md. Seven lots including one lot of stems were collected. Each lot was divided into two portions; one portion was ground to a fine powder for use in the direct tanning of sheepskin skivers, and the other was used for leaching experiments and the preparation of sumac extract for tanning tests. The extracts were prepared by a countercurrent leaching system in which the leaves in each vat were steeped with water for five 20-minute periods in a water bath maintained at 95°–98° C. The leaching efficiencies, or ratios of tannin extracted to tannin in the original leaves, ranged from 67.0 to 80 percent for the leaves dried in the various ways. The purities of the extracts obtained ranged from 50.0 to 69.5. No specific relation between either leaching efficiency or purity of extract and the method of drying was evident in these tests, with the possible exception that samples allowed to heat spontaneously apparently were modified so that tannin was more easily extracted.

In connection with the cooperative program on growing of sumac and the development of high-tannin strains, the tannin contents of 111 samples of sumac from selected strains of two species under cultivation by the Bureau of Plant Industry, Soils, and Agricultural Engineering at Beltsville, Md., were determined. The *Rhus glabra* samples contained from 27.4 to 35.6 percent of tannin on a moisture-free basis; the *Rhus copallina* samples contained from 29.0 to 44.5 percent. These results show that high-tannin strains of both species are available for expanded planting.

Scrub oak barks.—Analyses of additional samples of *Quercus laevis* and *Quercus cinerea* barks from small-diameter trees, limbs, and trunks showed that the average tannin content was highest in bark from 1- to 2-inch-diameter trees and from limbs. The value for bark from 1- to 2-inch-diameter trees of *Quercus laevis* was 11 percent; that for bark from *Quercus cinerea* limbs was 8.2 percent. These results show that bark from small trees, tops, and branches is acceptable as a source of tannin. Laboratory leaching tests produced liquors from which powdered tanning extracts were prepared by vacuum evaporation. The extract from *Quercus laevis* bark showed 58 percent tannin and 61.9 purity; that from *Quercus cinerea* bark showed 46 percent tannin and 55.3 purity.

Nutshells.—Additional samples of nutshells were examined for tannin. In three samples of pecan shells the tannin content of the outer shell ranged from 0.9 to 1.2 percent and that of the liner from 32.7 to 43.6 percent. A sample of California walnut shells showed 1.5 percent in the outer shell and 10.2 percent in the liner. An industrial plant for producing tanning extract from pecan shells is now in operation.

VEGETABLE-TANNED LEATHER INSOLES IMPROVED BY RETANNING WITH ALUM

Military and civilian shoes frequently fail in service because of deterioration of the vegetable-tanned insole leather. For developing a leather that should resist deterioration when used as insoles, consideration was given by the Eastern Regional Laboratory to the use of alum-retanned vegetable-tanned leather. In the preliminary tests

the leathers were not completely alum-retanned, but in an actual wear test conducted by the Office of the Quartermaster General these leathers used as insoles showed 68 percent fewer failures than did vegetable-tanned insoles. Later an alum-retannage process was developed by which vegetable-tanned leather of insole weight could be successfully retanned with alum so as to contain over 3 percent of fixed aluminum oxide. The tannage involves treatment of the leather with an aluminum sulfate solution which has been stabilized by the addition of sodium acetate and adjusted to the desired basicity by the use of sodium carbonate. The aluminum oxide is fixed by the subsequent neutralization of the leather. This process can be carried out in the tannery in ordinary tanning drums or rocker vats.

Alum retannage increases the resistance of the leather to damage by moist heat and molds. It also decreases water absorption and elongation and thus produces a leather that is more stable as to area. More uniform distribution and fixation of aluminum oxide were attained, and retanned leathers containing from 3 to 5 percent of aluminum oxide were prepared. These leathers withstood boiling water for 3 minutes without change in area and showed shrinkage temperatures ranging from 108° to 117° C. Leathers prepared in the laboratory by this procedure have been cut into insoles and are awaiting actual wear tests in shoes to establish their serviceability.

ADVANCES MADE IN THE CHEMISTRY OF FATS AND OILS

Previous research has shown that when fats and oils are subjected to certain processing, refining, and catalytic treatments, and also when they undergo oxidative deterioration, there is a rearrangement or shift in the double-bond system of the unsaturated acid components to yield more reactive conjugated fat acids. Treatment with alkali under certain standardized conditions produces extensive conjugation which can be measured spectrophotometrically. This reaction and measurement have been made the basis of an extremely important method for analyzing fats.

Investigation of the mechanism of this type of double-bond shift led to the development of a new method for fractionating and isolating pure natural unsaturated acids of fats based on the principle of chromatography. A study of the conjugated isomers produced by alkali treatment of the pure natural fat components and of their specific absorption coefficients made possible the establishment of more accurate absorption values for use in the spectrophotometric method of analysis. Previous values established for these acids were obtained from studies on unsaturated acids prepared by bromination and debromination techniques which result in the formation of geometrical isomers that have significantly different absorption characteristics than those of the natural acids.

From the results of the study of double-bond shifts in fat acids, theoretical concepts were developed which should prove valuable in research on other related mechanisms involving double-bond shifts.

Observations on the reactivity of the various conjugated isomers formed by alkali treatment of unsaturated acids may have practical application to drying-oil problems.

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ASCORBYL ESTERS PRODUCED COMMERCIALLY FOR PRESERVING LARD

The fatty acid monoesters of l-ascorbic acid (vitamin C), which were first prepared by the Eastern Regional Research Laboratory scientists for use as lard preservatives are closely related structually to l-ascorbic acid. It was assumed, therefore, that these new compounds would have antiscorbutic activity. Additional investigation confirmed this assumption, and, furthermore, demonstrated that l-ascorbyl palmitate and l-ascorbic acid are equivalent, on a molar basis, in antiscorbutic activity. Chronic toxicity studies over a 2-year period showed that l-ascorbyl palmitate is not toxic in a concentration of 0.25 percent or less of the total diet of rats, and studies of subacute toxicity extending over a 9-month period showed that l-ascorbyl palmitate is not toxic in a concentration of 2 percent of the total diet of rats. This information suggests several interesting pharmaceutical uses for the ascorbyl esters, in addition to their utility as synergists in antioxidant mixtures containing lecithin and tocopherols, which was mentioned in previous reports.

Commercial interest in the fatty acid monoesters of l-ascorbic acid resulted in applications from seven industrial firms for licenses to operate under U. S. Patent 2,350,435 (1944), entitled "Derivatives of Ascorbic Acid," which was issued to Bureau scientists and assigned to the Secretary of Agriculture. Furthermore, it is reported that the commercial production of l-ascorbyl palmitate by at least one of these firms is already under way.

ANALYSIS OF CASEIN COMPONENTS COMPLETED

It was reported last year that alpha-casein and beta-casein, the main components of casein, differ in their content of certain amino acids. During the year determinations of nine additional amino acids were made, and the combined results showed the following comparative distribution of amino acids: (1) Lysine, arginine, tyrosine, tryptophan, cystine, glycine, isoleucine and aspartic acid are present in higher concentration in alpha-casein; (2) serine, methionine, valine, leucine, phenylalanine and proline are found in higher concentration in beta-casein; and (3) histidine, threonine, glutamic acid, and probably alanine occur in equal concentration in alpha- and beta-casein. A summation for each protein of the nitrogen in its constituent amino acids, together with the amide nitrogen, approximated 100 percent of the total nitrogen. It is apparent, therefore, that a substantially complete picture of the amino acid composition of each fraction has been obtained and that if other amino acids are present, they occur only in small proportions.

An important difference in the properties of alpha- and beta-casein, which was utilized at the Eastern Regional Laboratory for the separation of these components of casein, is the greater solubility of beta-casein in aqueous alcohol. The difference in solubility can now be understood in terms of the polarity of the amino-acid residues in the proteins, because from the analytical data the numbers of ionic polar groups and nonpolar groups per unit weight of alpha- and beta-casein were computed, and it was found that beta-casein contains about 14 percent fewer ionic polar groups and about 29 percent more non-

polar groups than alpha-casein. Similar correlations between properties and composition should be valuable in continued work on the utilization of casein and its components.

CASEIN BRISTLES MADE MORE DURABLE

Casein bristles hardened with quinone are suitable for making brushes to be used in the dry condition or in the presence of nonaqueous solvents. In the presence of water, however, such brushes become soft and lose their form. Further work at the Eastern Regional Research Laboratory has been directed toward improving the stability of casein bristles, particularly their resistance to soaking in soapy water. It was found that for brushes of the twisted-in-wire type, casein bristles hardened with formaldehyde are superior to the quinone-hardened bristles in retaining their shape and flexibility after repeated use in water. The addition of small quantities of heavy metal salt further improved the stability of these brushes in water. Mercuric chloride was more effective in this respect than other heavy metal salts. The application of these findings has resulted in a less expensive method for producing casein bristles of superior durability.

MORE VOLATILE FRUIT FLAVORS RECOVERED

The identification of the chemical constituents of apple essence has been completed. The principal constituents, together with the relative amounts of each class are: Alcohols, 92 percent (including methanol, ethanol, *n*-propanol, isopropanol, *n*-butanol, isobutanol, *d*-2-methylbutanol, and *n*-hexanol); aldehydes, 6 percent (including acetaldehyde, *n*-caproaldehyde, and 2-hexeneal); and esters, 2 percent (principally methyl butyrate and ethyl caproate). Methanol, ethanol, isopropanol, *n*-butanol and formic, acetic, propionic, butyric, and *n*-caproic acids were identified as components of other esters. Altogether, these compounds were present in the original apple juice at a total concentration of about 50 parts per million. It seems unlikely that a synthetic apple flavor having the true aroma of fresh apples can be prepared simply by combining the synthetic compounds. A combination of all the known constituents in proportions roughly approximating those in the essence has an odor only slightly suggesting apples.

The presence of naturally formed ethyl alcohol in apple essence recovered from fresh apple juice has temporarily retarded industrial production of the essence in this country, because a Federal tax of $9.00 per wine gallon is imposed when the alcohol concentration is 0.5 percent or over. Furthermore with this alcohol content the operation of essence-recovery equipment falls under the other restrictions imposed on distilleries by the Bureau of Internal Revenue. Although in experimental work it is possible to produce 150-fold apple essence with an alcohol content below the taxable limit, the method originally proposed by the Department of Agriculture is not practicable for this purpose. Research has therefore been actively pursued to obviate the difficulty.

An 800-fold essence has been prepared having the full flavor characteristics of 150-fold essence, but, being more than five times as strong, its value would be proportionately higher, and hence the burden of the tax proportionately lower. It has also been found practical to

produce a good essence of approximately 150-fold concentration and having less than 0.5 percent ethyl alcohol, if only 3 percent of the juice is vaporized in recovering the essence instead of the usual 8 or 10 percent. This reduction in juice vaporization may prove to be a practical means of avoiding the tax complications. Other methods for eliminating ethyl alcohol from the product are under investigation.

The recently constructed portable essence-recovery unit, which has a capacity of 10 gallons per hour of juice, was used in some field experiments under a Research and Marketing Act project on the recovery of volatile flavor constituents from the juices of strawberries, peaches, blackberries, Youngberries, rhubarb, huckleberries, and grapes of the Delaware, Moore Early, Niagara, and Worden varieties. In almost all cases the volatile aroma was more difficult to recover than that from apple juice. For most of the fruits about 20 percent of the juice had to be vaporized to evolve the aroma in contrast to about 3 percent for apples. The essence from strawberries, peaches, huckleberries, rhubarb, Moore Early grapes, and Worden grapes had strong characteristic aroma. Niagara grape and Delaware grape essences were strong and fragrant but lacked full grape character. Blackberry and Youngberry essences had less of the full berry flavor but contained the "top note" and in concentrated form were recognizable. Some pectin-gum candies and jellies prepared with these essences as the sole source of fruit flavor were favorably considered by a large taste panel. The products made with strawberry and Youngberry essences were especially palatable.

DEHYDROFREEZING PROCESS APPLIED TO FRUITS AND VEGETABLES

The Western Regional Research Laboratory has continued work on the dehydrofreezing process for fruits and vegetables, which includes partial dehydration to reduce weight and volume, followed by quick freezing and ordinary frozen-food storage. The potential economic advantage of this process in comparison with quick freezing lies in lower transportation and storage costs. Thus far, the dehydrofreezing process has been studied in detail as applied to apples and peas and to a lesser extent as applied to apricots. Exploratory work has been done on dehydrofreezing of other fruits and vegetables, such as peaches, cherries, carrots, and white potatoes. Problems considered recently have dealt largely with effects of variations in processing techniques on quality of the product.

Further experiments with peas showed that the air temperature can be varied between 160° and 200° F. without sensible effect on quality of product when drying is carried out to 60-percent weight reduction, based on weight of fresh shelled peas. They also showed that for dehydrofreezing it is feasible to dry, in one batch, peas ranging in size from 3 to 6. When peas of this size range were dried together the dehydrofrozen product seemed to be just as good as when two separate batches made up of the larger or smaller sizes were dried separately.

Although blanching temperatures and times have little effect on either drained weight or volume of ordinary cooked frozen peas, they do exert a measurable effect on these properties in the case of peas dehydrated to 50-percent weight reduction before being frozen. In order for the drained weight and volume of cooked dehydrofrozen peas

to equal those of the cooked control sample it was necessary to blanch at 212° F. for 50 percent more time than was required for a negative peroxidase test. Lower blanching temperatures (down to 190°) led to drained weights that ranged from 5 to 15 percent lower than those of the control, depending on duration of blanch (up to 300 percent of adequate time).

Results of a storage experiment showed that dehydrofrozen pea samples lost little of their eating quality during 1 year of storage at −10° F. At the end of this period, dehydrofrozen peas were about equal in quality to ordinary frozen peas. Another storage experiment, initiated to learn the relationship between processing conditions and stability of dehydrofrozen peas in storage, indicated that overblanching had no deleterious effect and that the permissible extent of weight reduction lies in the vicinity of 50 percent. The principal disadvantages of more extensive dehydration are deterioration in color and reduction in capacity for rehydration. Greater leaching losses comprise the obvious disadvantage of overblanching.

Recent work on dehydrofrozen apples was concerned largely with details of the sulfiting procedure for inactivating enzymes. By adjustment of factors controlling concentration of sulfurous acid in the dipping bath, retention time for slices in the bath, and drying conditions, it was found feasible to reduce the drying times previously found necessary.

Delicious, Gravenstein, Winesap, and Jonathan varieties of apples were compared with regard to suitability for dehydrofreezing. Although one season's work does not warrant final conclusions, it has offered strong evidence that the Gravenstein variety is the best suited, because it is most easily rehydrated. For use in a pie, dehydrofrozen Gravenstein apple slices require only the addition of boiling water, whereupon they are ready for the pie shell. In contrast, the harder-textured varieties require a cooking period in addition to that received during baking. Results of preliminary experiments offer some promise that this extra cooking period can be avoided by substituting blanching in hot, humid air for the sulfite treatment. Steam blanching is not suitable for apples because of serious leaching losses.

According to tentative cost estimates based on procedures used in the experimental studies, the processing cost in dehydrofreezing was somewhat greater than in freezing, but the lower packaging, freezing, storage, and distribution costs in dehydrofreezing should result in an over-all saving of 20 percent to the user.

PROGRESS MADE IN FREEZING PRESERVATION OF FOODS

At the Western Regional Research Laboratory a comprehensive program on freezing preservation of fruits, vegetables, and poultry products is in progress, in accordance with expressed needs of the food-freezing industry.

Fruits.—In the freezing preservation of fruits, improvement in control of enzymatically induced oxidative discoloration continues to be needed. A method for estimating the tendency of samples toward discoloration (consisting of analytical determination of catecholase and catechol tannin) was applied to over 50 samples of peaches representing different varieties and localities. Results indicate that samples containing 40 milligrams or less of catechol tannin per 100 grams of

prepared fruit are substantially free from tendency to brown upon thawing of the frozen product. The testing was done in cooperation with the Washington and California State agricultural experiment stations. It is believed that the method will prove useful in plant breeding as well as in the selection of raw fruits for freezing.

Two rapid methods that can be used by the orange-juice industry for routine differentiation of coliform bacteria into fecal and nonfecal varieties were tested on a large number of orange-juice samples. One gave in 93 percent and the other in 97 percent of the cases results that agreed with those obtained by the accepted but longer standard method. Though the short methods have not been adopted by the American Public Health Association, they will be useful in the control of orange-juice production, because no other tests or formal standards have been established for this product.

Vegetables.—The temperature of the steam used for blanching peas was found to affect the tendency of pea skins to rupture. When the operation was carried out at 212° F. the number of ruptured skins was relatively large and increased as blanching time was extended. When the temperature of the steam was reduced to 190° F. by addition of air, rupturing of skins practically ceased. The peas referred to were processed immediately after picking while the skins were tender; after vining the skins of raw peas toughen with consequent increase in their resistance to rupture. Loss of solids by leaching increased with blanching time and this increase was greater at 212° than at 190°.

Studies were initiated to determine if the use of radio waves for inactivation of enzymes in fruits and vegetables before freezing would result in products of better flavor than when the raw material was scalded by steam or hot water. Tests on shelled peas showed that adequate diathermic heating is obtained at 30 megacycles per second, but the irradiation required an unduly long time in order to minimize arcing between adjacent pea surfaces. Measurements of electrical conductivity at low radio-frequencies showed that the probable cause of arcing between peas is the disparity between the conductivity of peas and that of water and air films between peas. Theoretical considerations and measurements of the electrical impedance of microorganisms (yeasts) indicate that arcing would be less at higher radio-frequencies because lower voltages would be needed for the heating. Efforts are being made to test this prediction and to examine further the usefulness of radio waves for blanching and pasteurizing foods, especially juices and purees.

Preliminary experiments showed that the rate of freezing peas had no effect on texture of the skins, but that variations in rate of freezing did affect the quality of frozen asparagus substantially. When freezing was accomplished in 10 to 20 minutes the rigidity, skin texture, and flavor of asparagus tips were definitely superior to the corresponding qualities in similar material frozen in 6 hours.

Poultry.—It has long been supposed that one of the principal factors limiting shelf life of properly prepared frozen turkeys is rancidification of the fat. Experimental packs were studied in cooperation with the Utah Agricultural Experiment Station to determine whether alfalfa meal in the diet influences the tendency toward rancidification. Subjective examination after storage indicated that alfalfa meal in the diet did not influence rancidification of the fat in the frozen tur-

keys. Another experiment concerned the influence of length of chilling period between evisceration and freezing on the tendency of turkeys to become rancid during frozen storage. After 13 months in frozen storage at −10° F., subjective examination of turkeys chilled for various periods up to 30 hours after evisceration failed to show objectionable rancidity in any, although chemical tests indicated increased peroxide formation (rancidification) in the fat of turkeys exposed to chilling conditions (36° F.) for the longer periods. The results indicate that rancidity from this cause can be controlled and that, as is generally recognized, chemical tests must be corroborated by subjective examination if results are to be significant.

Recent years have witnessed variable surpluses of turkeys that are individually too large for single-family consumption. Their profitable disposition has become a problem of special interest because it appears certain that production of large turkeys is more efficient in terms of labor and feed than the production of small turkeys. Turkey halves and quarters as well as turkey parts, steaks, and precooked turkey dishes have evident possibilities. Experiments are in progress on freezing preservation of turkey steaks and precooked dishes. Steaks prepared both by transverse sawing and by deboning and "knitting" the meat in a cube-steak machine have been subjected to storage tests. Results obtained thus far have indicated that the greater surface exposed renders them more sensitive to oxidation and desiccation than is the whole carcass. Extra-good packaging is therefore required for such products.

An advantage of turkey steaks would be the short cooking time required, but the steaks are likely to be deficient in turkey flavor. Economical utilization of parts not suitable for steak preparation would be necessary. An excellent loaf prepared from ground neck meat is undergoing tests for stability. Similar work is proceeding on the commercial formulation, preparation, and storage of precooked dishes such as turkey a la King, in which flavor fading has proved a problem.

Equipment.—In a cooperative study on an air-blast freezer in a commercial plant it was shown that actual time required to reach 0° F. varied among individual packages of lima beans in 12-ounce packages from 4½ to 8¾ hours. The principal cause of variation proved to be lack of uniform air flow in various parts of the equipment. To facilitate testing of this kind as well as for the testing of experimental equipment, an improved anemometer, operating on the hot-thermocouple principle, was designed and built. The anemometer is convenient in use, yields results of excellent precision with regard to air velocity, and will assist in the evaluation and development of air-blast freezers.

MORE LEARNED ABOUT DEHYDRATION OF VEGETABLES

Despite the decline in commercial vegetable dehydration following the war, dried vegetables are sufficiently important to justify continued research. There are as yet no adequate criteria for raw-material selection; neither is there sufficient information on procedures to show unequivocally the direction in which improvements can be made. Moreover, much remains unknown with regard to deteriorative changes during storage, particularly as these changes are related to composition of raw material.

Experiments at the Western Regional Research Laboratory on susceptibility of potato to discoloration or so-called heat damage during dehydration indicated that it varies greatly with the moisture content of the potato mass. Preliminary results showed that in vacuum drying the maximum rate of heat damage occurs when the moisture content is between 10 and 15 percent, whereas in air drying the maximum rate occurs when the moisture content is between 40 and 60 percent. When the potato mass was dried in air a small quantity of water-soluble brownish substance, together with an insoluble grayish substance, was formed, whereas only the former type of colored substance appeared when potato was dried in a vacuum. The results of these experiments suggest that it might be possible to decrease heat damage by first reducing moisture content to about 18 percent by vacuum drying and then finishing the dehydration in air.

Experiments with two varieties of potatoes, each being divided into lots which were stored at different temperatures to get relatively high and low sugar contents before being dehydrated, showed that the sugar content is a dominant factor in nonenzymic browning of dehydrated potatoes stored at a high temperature (120° F.).

Evidently the brown substance formed during dehydration and also during storage at a high temperature is an addition product of sugar with protein components, examples of which are well known to organic chemists.

It was previously shown by the Bureau of Plant Industry, Soils, and Agricultural Engineering that excessive browning of potato chips during frying was related to a relatively high sugar content which could be caused by too low a temperature during storage.

Extended storage experiments were made with the dehydrated products from Katahdin and Green Mountain potatoes to determine the influence of variety and storage conditions on quality. Dehydrated potato packed in cans from which the air was displaced by nitrogen before being sealed kept in very good condition, but all samples packed in cans without displacement of air developed sufficient off-flavor to render them undesirable. After being stored for 13 months, dehydrated potato containing 7 percent moisture (representing ordinary trade practice) and dehydrated potato containing 3 percent moisture (achieved by in-package desiccation) showed no difference in eating quality. Dehydrated potato stored at 33° F. was only slightly better than that stored at 75° F. No difference with regard to eating quality seemed to exist between the dehydrated products of Katahdin and Green Mountain potatoes. There was no difference in the keeping quality of dehydrated-potato samples prepared from light and heavy potatoes separated by brine flotation and representing high-starch and low-starch potatoes respectively.

Some of the results of the storage experiments, corroborating earlier findings at the Western Regional Laboratory, indicated an advantage in packaging dehydrated potato in contact with nitrogen or other inert gas for storage at room temperature or below. Moreover, they showed that a very low moisture content resulting from in-package desiccation, which is advocated for high-temperature storage of dehydrated potato, will not prove deleterious to the product when held at low temperature. This latter point is noteworthy because certain products are known to become rancid or stale more rapidly as complete dryness is approached.

Nonenzymic browning of dehydrated cabbage and other vegetables is commonly retarded by use of sulfur dioxide or sodium sulfite, but the sulfurous acid in these chemicals forms unstable compounds in the product and imparts objectionable taste unless the dosage is very low. To aid in estimating the minimum sulfite dosage needed for protection of a dehydrated vegetable under specific conditions of transportation and storage, measurements were made of the rate of sulfite disappearance from dehydrated vegetables stored under a wide range of conditions.

BROWNING OF DEHYDRATED FOODS EXPLAINED

Many dehydrated foods turn brown during prolonged storage. The causes have been obscure, although it has been realized that some browning might be due to the reaction, first described by Maillard, between reducing sugars and proteins (amino compounds). In a detailed study of this reaction, as exemplified by glucose and protein, which was carried out by the Western Regional Research Laboratory at the request of the Quartermaster Corps Food and Container Institute, it was discovered that browning occurs in solution if the concentrations of protein and glucose are high enough. Browning is hastened by increasing temperature and also by increasing alkalinity. Addition of sulfite in traces did not decrease the rate appreciably, but sucrose in fairly high concentration decreased the rate, as observed in British investigations on dried eggs.

Studies on the protein groups involved showed that amino groups are essential. Proteins turned brown at rates proportional to the amount of amino nitrogen present. When amino groups were eliminated, for example by selective acetylation, no browning occurred. Guanidyl groups and, to a lesser extent, indole groups participated in secondary reactions.

The brown reaction product of glucose and blood-serum albumin, used as a model protein, was insoluble at pH 4.3 (its isoelectric point), whereas the original protein was soluble at that pH. Solutions at pH 7 (neutral point) were not coagulated by heat. The protein-sugar compound was attacked by enzymes, but at a slower rate than in the case of unreacted serum albumin. Both microbiological assays and chemical studies showed that the former compound contains less lysine and arginine (amino acids) than does the latter.

One practical conclusion from this study, which has immediate application, is that dehydrated foods should be stored at as low a temperature as possible to avoid undesirable discoloration.

FRUIT AND VEGETABLE WASTES UTILIZED THROUGH BIOLOGICAL CONVERSION

The continued need for increased supplies of protein feed supplements and the increasingly urgent problem of providing better means of waste disposal emphasizes the need for continued research on conversion of fruit and vegetable wastes into useful products. The year's activities along this line at the Western Regional Research Laboratory included: (1) Conversion of fruit wastes, particularly pear, into feed yeast, (2) use of asparagus juice as a medium for micro-organisms, particularly *Bacillus subtilis*, which produces the antibiotic subtilin,

and (3) growth of mushroom mycelium by vat fermentation on concentrates prepared from fruit and vegetable residues.

Previous reports have indicated successful operation of a pilot plant for the production of torula yeast from pear juice. The application of this process on a commercial scale is now largely dependent upon the development of an economical, continuous press for recovery of the juice from pear and other fruit wastes. Studies during the past year on the pressing characteristics of pear waste showed that no commercial press now available has operating characteristics suitable for this material. Preliminary studies were made of the kind of press needed, and a contract for development and construction of a pilot-plant juice press was negotiated under an RMA project.

Extension of nutritional studies on the growth of *B. subtilis* made possible production of subtilin on a small pilot-plant scale in which high yields were obtained. A simplified method for isolating and purifying the antibiotic by salt precipitation rather than by petroleum-ether extraction yielded a product of high purity and biological activity.

Low solubility of subtilin in blood serum makes parenteral administration difficult and limits internal use of this antibiotic. In efforts to obtain more soluble derivatives two modified subtilins were prepared. One was a methylated derivative having 45 times the solubility of the original subtilin and equal biological activity. The other was a subtilin-pectin complex which also showed enhanced solubility. Subtilin and the derivatives mentioned are now being evaluated for their biological activity against a number of pathogens (tuberculosis, bovine mastitis, trichomoniasis, anthrax, amoebiasis, and rickettsial, streptococcal, staphylococcal, and pneumococcal infections) in the laboratories of 15 Federal and State institutions and pharmaceutical manufacturers. Preliminary results in a number of cases have been promising.

Discovery at the Western Regional Laboratory that the mycelium of the commercially grown mushroom *Agaricus campestris* can be obtained by submerged fermentation of juices prepared from fruit and vegetable wastes has opened a new field of research with many potentialities. The preliminary results suggested that a palatable product, suitable for use as a flavoring agent in meat dishes or as a substitute for mushrooms in soups, can be grown on various sugar-containing media with yields equal to those of feed yeast. This discovery also suggests that the vat-grown mycelium may possess advantages over commercial spawn for starting mushroom beds, because the bed can be thoroughly drenched with the inoculum, which should insure a faster "run" of the mycelium. Commercial growers and users of mushrooms have expressed great interest in this development.

INDUSTRIAL USES FOUND FOR PECTINATE FILMS

Development of low-methoxyl pectin as a coating agent was continued at the Western Regional Research Laboratory by (1) improvement of methods of applying the coating and conditions for forming the film, (2) modification of properties of the film through incorporation of sugars, fungicides, or moistureproofing agents, and (3) cooperation with industry in application of pectinate films to candied and dried fruits, meat products, food bars, and seeds.

Initially the film was formed by dipping the object into a hot solu-

tion of sodium-calcium pectinate, cooling till the liquid coating gelled, and then drying. A new procedure having many advantages has been developed. It consists of dipping the object in a solution of sodium pectinate at room temperature, immersing in a solution of calcium chloride, and then drying. Among the advantages are a wider latitude of temperatures for applying the coating, and hardening of the film with calcium chloride, which prevents adhesion between individual pieces. A machine was designed and constructed for coating articles by this method.

Attack of the pectinate film by micro-organisms whenever enough moisture and nutrient are present to provide a good medium for growth is one of the disadvantages to be overcome. One promising method is to increase the total soluble-solids content to at least 70 percent. This method was used with candied fruits and some dried fruits without loss of glossy appearance, and the coated products had the desired nonsticky, free-flowing characteristics.

The moisture permeability of pectinate film, like that of ordinary cellulose film, is high. Although high moisture permeability is advantageous for some uses, a low moisture permeability is desirable for others. Moisture permeability was reduced to one-tenth of the original value, and further progress in this direction is anticipated.

Cooperation with a large producer of candied-fruit mixtures resulted in the development of an attractive free-flowing product adapted to mechanical packaging, and samples are undergoing final storage tests. Dates of the soft variety were coated experimentally, and one date producer and packer planned to apply this method in coating a portion of his pack during the next season. Sufficient low-methoxyl pectin and directions for applying it as a film on concentrated food bars, to prevent their adherence when packed and to eliminate need for removal of a wrapper before eating, were supplied to one manufacturer. Six thousand bars were coated and given a field trial. The result was so promising that 100,000 bars are to be coated for a more extensive test.

Another possible application of pectinate film is as a carrier for fungicides, vitamins, antioxidants, and other chemical substances. Hybrid sweet corn seed has a tender skin, readily ruptured by mechanical shelling, and is therefore susceptible to fungal attack when planted during cold, damp weather. For protection, such seed has been coated with a pectinate film containing commercial seed disinfectants and, in one case, with copper pectinate. In tests carried out by a seed company under adverse conditions, germination was increased in some cases from 37 percent for untreated seed to almost 60 percent for treated seed. However, the rate of sprouting appeared to be decreased; so further experiments are in progress to learn if this difficulty can be overcome by using thinner pectinate films.

MINOR PROTEINS OF EGG MAY HAVE MEDICINAL USES

During the war years the Western Regional Research Laboratory investigated the nature of egg proteins as part of an international coordinated program designed to reduce deterioration in dehydrated whole eggs. One phase of this investigation that has been continued is on the biological activities of several of the minor proteins. These activities are important with regard to possible nonfood uses for egg constituents, because compounds possessing special biological action

can command prices that would make profitable the use of edible egg materials for nonfood purposes.

New or improved methods have been developed for isolating three biologically active proteins from eggs. These are: Lysozyme, which dissolves certain bacteria; conalbumin, which binds iron so strongly that it limits biologically available iron; and ovomucoid, a selective inhibitor for trypsin but for no other enzyme. A method has also been developed for isolating from egg yolk a new protein, phosvitin, of a type not previously known to exist. Studies have recently been initiated on avidin, which binds biotin but no other vitamin. An elucidation of the mechanism through which it binds biotin appears important, because this protein is reported to retard the growth of some kinds of tumors.

The distinguishing feature of phosvitin is its large proportion (10 percent) of phosphorus chemically bound as phosphate. Some of the properties of isolated and purified phosvitin were learned but whether it is biologically active remains to be determined. Phosvitin commands considerable biochemical and embryological attention because of the important role that phosphorus plays in metabolism.

Crystalline lysozyme is now being prepared commercially from egg white by the method discovered in the Western Regional Laboratory. The present demand seems to be for experimental use in medical research.

Conalbumin, which the Western Regional Laboratory identified as the antibiotically active iron-binding component of raw egg white, is similar in all physicochemical characteristics to a recently crystallized iron-binding globulin from human blood plasma. The latter plays an important metabolic role in man. Therefore it seems possible that if the tendency of conalbumin to make persons sensitive to egg protein could be abolished this protein might well find medicinal use to compensate for pathological deficiencies of the corresponding plasma fraction.

Much was learned about the properties of ovomucoid, the trypsin inhibitor of egg white. The nature of the combination of ovomucoid with trypsin was studied by determining the degree of enzyme inhibition that occurs with derivatives of ovomucoid or trypsin in which one or more of the types of chemical groupings in the protein have been blocked by acetylation, esterification, or other chemical means.

Trypsin in which amino groups had been acetylated still retained enzymatic action which was not inhibited by ovomucoid; therefore it was possible to determine trypsin as acetyl-trypsin in an acetylated mixture of trypsin and ovomucoid. In this way it was demonstrated that trypsin is absent from egg white, which answers a question that has been raised repeatedly in the last 20 years.

Ovomucoid is one-third as active as the purified (derived) trypsin inhibitor from lima beans and is half as active as the crystalline soybean trypsin inhibitor. The latter is now being manufactured commercially and appears to resemble ovomucoid rather than the lima bean inhibitor in its manner of combination with trypsin. The inhibitors isolated from beans contribute to the growth-inhibition action of unheated bean meal in the diet. Some trypsin inhibitors have blood-anticoagulant properties and may prove useful in medical practice as anti-coagulants.

EGG DETERIORATION NOT CAUSED BY NATURALLY OCCURRING EGG ENZYMES

For a long time food processors and technologists have asked whether egg enzymes cause deterioration of eggs and egg products. An investigation at the Western Regional Research Laboratory has shown that uncontaminated eggs contain such small amounts of enzymes that deterioration of shell, liquid, frozen, or dried eggs is most likely due to nonenzymic factors or to microbial contaminants. The investigation included determinations of specific enzymes that might cause observed types of deterioration and examination of sterile egg products for possible enzymically catalyzed autolytic changes.

Small amounts of the enzymes peptidase (erepsin), phosphatase, catalase, amylase, and esterase (active on low-molecular-weight esters) were found in eggs, but tests for trypsin, polyphenol oxidase, cytochrome oxidase, peroxidase, proteinase, and lipase (active on fat-like compounds containing fatty acids with six or more carbon atoms) were negative. Although definite evidence of activity by several enzymes was found in eggs, there was little or no enzymically catalyzed autolysis in sterile eggs. In fact, materials upon which enzymes are capable of acting are either absent from eggs or exist there in insignificant quantities. In addition to their practical value, these results contribute to general knowledge of the biochemical characteristics of pre-embryonic materials.

In contrast with these results, enzymes of contaminating bacteria have been shown to accelerate deterioration in dried eggs, even when the bacteria are dead. Liquid-egg mixture was inoculated with a culture of *Pseudomonas fluorescens* and immediately dried from the frozen state. Although the bacteria did not grow either during processing or in storage, the dried products deteriorated at a markedly greater rate than similar products dried without added bacteria. The fact that more than 99 percent of the added organisms died during processing explains the previously known fact that egg powders prepared from liquid egg of poor sanitary history deteriorate rapidly during storage, even though the count of viable bacteria is low. The observations reemphasize the importance of strict sanitary practices, even though growth does not occur and viability is lost in the final product.

The conclusion from this completed investigation is that egg deterioration may be due in part to enzymes of contaminating microorganisms, but not to naturally occurring enzymes.

FOOD-POISONING ORGANISMS ABSENT FROM MOST LOW-MOISTURE EGG POWDERS

The Microbiology Research Division continued and extended its bacteriological investigations on dried egg and other egg products to provide information needed by egg processors to improve the quality of their products. As expected, dried egg is finding extensive peacetime use by the civilian population of the United States and other countries.

Some of the investigations on high-moisture and low-moisture egg powders were carried on in cooperation with the Quartermaster Corps of the United States Army. Many samples of egg powder contain-

ing from 4 to 6 percent moisture were found to be contaminated with one or more types of *Salmonella* (food poisoning) organisms. A few samples contained as many as five or six *Salmonella* types.

Comparative studies on high-moisture and low-moisture egg powders revealed that egg powder dried to a moisture content below 2 percent is less likely to contain viable *Salmonella* organisms. Such organisms were absent from 94 percent of the low-moisture egg powders examined.

The findings from the bacteriological studies of dried egg served as a basis for the development of a Research and Marketing Act project in cooperation with the University of Chicago to investigate the pathogenicity for humans of the *Salmonella* types isolated from dried egg.

NEW AND IMPROVED PRODUCTS DEVELOPED FROM WESTERN FRUITS

During the past 3 years, the Laboratory of Fruit and Vegetable Chemistry in Los Angeles, Calif., has carried on cooperative work with the California Desert Grapefruit Industry Board and the Arizona Grapefruit Program Committee on the preparation of uniform-quality canned grapefruit juice by adjustment of sugar-acid ratio with sugar and carbonate salts. This work has now been completed. Although it has been demonstrated that superior-flavored grapefruit juice can be produced throughout the canning season, industry's acceptance of juice modification has been slow because of labeling requirements. However, as a result of this work, there is a growing tendency among processors of desert grapefruit to limit their canning season to the months when natural sugar-acid ratio is such as to give an acceptable canned juice.

Studies on the keeping quality of unpasteurized, refrigerated orange juice for distribution in and around Los Angeles, and to more distant points by rail, have indicated that a superior fresh juice can be produced from good-quality fruit that has been thoroughly washed and dried by reaming carefully to get as little peel oil as possible in the juice, which is then strained, deaerated, quickly chilled to 30° F. by passing through a heat exchanger, filled into containers without headspace, and held at 30° F. until consumed. Refrigerated juices thus prepared have retained good flavor and shown no loss of vitamin C after 21 days' storage. In the case of deaerated juices held longer than 7 days, more of the fresh flavor was retained, and the desired cloudiness was better stabilized, than in undeaerated samples under similar conditions. The improved methods of processing unpasteurized, refrigerated orange juice have led to marked increase in production and distribution of this product in southern California, and many carloads of juice have been shipped as far as New York City with good results.

Results on preservation of citrus-fruit purees by freezing have shown such promise that one processor in southern California packed enough Valencia orange puree last season to supply five large ice-cream companies. This product was prepared by running whole washed oranges through a machine which cut and pressed the fruit to yield about 50 percent of pulp and juice mixture (puree), adding dry sugar in the proportion of 1 part to 5 parts puree, filling into 30-pound tins, and freezing. Contrary to general expectations, no

"terpeney" off-flavor developed in any of the test samples, despite the fact that they contained 0.75 gram of peel oil per 100 milliliters as contrasted with 0.006 gram of peel oil per 100 milliliters ordinarily found in strained orange juice. To insure rapid freezing of the puree in large containers, a rapid heat exchanger was used in some experiments to slush-freeze the freshly prepared puree prior to final freezing in the container. Frozen puree was prepared from Navel oranges, and orange sherbet made from the puree after 6 months' storage was judged to be of good quality. Some preliminary work was also done on the freezing preservation of lemon puree.

Growers of Navel oranges in California and Arizona have been unable to sell surplus fruit to processing plants because of the tendency of the juice to become bitter shortly after extraction. Several substances (the main one being limonin) in the peel of Navel oranges turn bitter when exposed to weak acids such as are present in the juice. Preliminary experiments, set up to determine whether the bitterness of reamed juice could be covered up by increasing sugar content, resulted in more palatable canned juice, but bitterness was still evident. Other experiments demonstrated that the bitter taste could be eliminated by sectionizing fruit and freezing in a sugar sirup. Undoubtedly the reason was that the sections were separated from the peel without contamination with bitter substances located there.

Work has been renewed on the processing and packaging of dates and the utilization of cull dates as byproducts. Cleaned dates have been coated with two water-soluble cellulose derivatives (methyl and hydroxy methyl ethers) in solutions containing 1 to 5 percent of dry material. This treatment eliminated the sticky surface of undried dates and produced a desirable glossy surface. Glycerine and propylene glycol were added in some experiments to act as plasticizers. The addition of either material was beneficial because it made the film coating softer and it gave a sweet taste to the film. Treated and untreated dates were packaged and stored at 85° F. for 45 days. There was little difference between samples with regard to amount of moisture lost, but the appearance of the treated dates was far superior to that of the untreated ones. The cost of applying these films amounts to less than 0.1 cent per pound of dates.

NEW INSTRUMENT MEASURES ETHYLENE IN CITRUS-COLORING ROOMS

Ethylene gas is used at low concentrations (10 to 200 parts per million parts of air) to develop the color of mature oranges which at the time of picking do not have the reddish-orange hue associated by many purchasers with the ripe fruit. Besides developing the color, ethylene stimulates respiratory activity of fruits. Since overtreatment with ethylene results in poor keeping quality and also destroys the "bloom" of prime fresh fruit, considerable economic loss now occurs from degrading oranges that are overtreated with ethylene. One reason for overtreatment has been the lack of a reliable field method for determining small amounts of ethylene in air. To overcome this difficulty the Western Regional Research Laboratory developed a small portable instrument by means of which the ethylene concentration in the air of coloring rooms at citrus packing houses can be readily measured.

The instrument is a modification of one recently developed for the determination of small amounts of carbon monoxide in air. A measured volume of air is passed over hot mercuric oxide and then over a hot strip of paper impregnated with selenium or selenium sulfide. Oxidation of ethylene in the air by the mercuric oxide results in the formation of metallic mercury which vaporizes and reacts with the sensitized paper to form black mercuric selenide or mixture of selenide and sulfide. The length of the blackening on the strip of paper is a measure of the ethylene in the air sampled. A determination can be made in a few minutes and does not require special training on the part of the operator. The instrument is useful for concentrations of ethylene above five parts per million.

ENZYME RESEARCH CONTRIBUTES TO SOLUTION OF USE PROBLEMS

Fundamental and practical research on the chemistry of enzymes and the nature of enzyme action in agricultural products was continued by the Enzyme Research Laboratory under projects supported by allotments from the appropriation items for Agricultural Chemical Investigations and the Special Research Fund. During the past year studies were made of the enzymes and enzyme reactions involved in the ripening of Navel oranges (including the frequent development of bitterness in such oranges and their processed juice), in the recovery of citrus fruit from damage by certain new insecticides, and in the decomposition of fats and fatlike substances in agricultural products.

Artificial ripening with ethylene (as well as natural ripening) was found to reduce the quantity of bitter substances formed in Navel oranges, but artificially ripened oranges developed other off-flavors. Attempts to avoid this are being made, but thus far without success. This year there was little tendency toward the development of bitterness in Navel oranges, so the results are not very conclusive.

Considerable progress was made in learning the chemical structure of the bitter substances, and this knowledge has helped to suggest the way they are formed. The development of bitter substances in Navel orange juice has been traced back to a nonbitter mother substance whose chemical structure can now be predicated in part. The problem is highly complex, but seems to be of great interest to the California citrus industry.

Continued studies on the changes that an orange undergoes during ripening (including some in storage), have given a much clearer picture of some details of the ripening process; they have discovered the presence of a member of the vitamin B_6 group (pyridoxal) in citrus fruits; and they have indicated that diisopropyl fluorophosphate (a war gas) and two of the new insecticides that are chemically related to it affect the metabolism of the fruit by the same chemical process that occurs in poisoned animals. But the effect on the fruit is only temporary, whilst that on the animal is likely to be permanent. This knowledge is important in citrus-fruit metabolism and perhaps even more so in connection with war-gas poisoning.

Work on starch-digesting enzymes resulted in the purification of a starch-splitting enzyme from malt so that 1 part will do the work of 5,000 parts of malt. It is not yet quite pure, but is being studied fur-

ther, together with the crystallized beta-amylase from sweetpotatoes mentioned in last year's report. The starch industry, the brewing industry, and the baking industry are anxious to know more about these enzymes.

One important finding was that the action of these starch-digesting enzymes is altered by some common constituents of plants. Traces of copper, the amino acid cysteine, and vitamin C appear to be such substances, according to present knowledge. To digest starch effectively the presence of such substances should be known, as well as means for preventing their interference.

In the course of work on the enzymic decomposition of fats and fat-like substances in agricultural products, a method was discovered for the synthesis of phospholipid compound cephalins. The process is practical and gives good yields. Because of its possible importance to industry, the method of synthesis has been patented. In addition to other uses, cephalins have been proposed as additives for improving lubricating oils. The process is of fundamental importance to agriculture because these substances, which are greatly involved in plant and animal growth, cannot be isolated in the pure state from natural sources and, therefore, could not be studied by themselves until they were prepared chemically.

ENZYME RESEARCH MAY LEAD TO BETTER CITRUS PRODUCTS

In order to produce satisfactory canned and bottled citrus products, it is necessary to harvest the fruit when it has maximum flavor and to know the possible causes for deterioration in flavor of the packed products and how to avoid or counteract them. A reliable test for maturity is needed to insure that the fruit be picked at the right time. Such a test might be based on the appearance or disappearance of particular enzymes, since enzymes activate the chemical changes that take place during ripening. Enzymes are also probably involved in the deterioration of citrus products after packing. Therefore the Enzyme Research Laboratory has participated in certain phases of the research under a Research and Marketing Act project which was recently inaugurated in this Bureau for the purpose of developing new and improved citrus products.

The California orange crop of the 1947–48 season was followed throughout its development in the hope of finding an enzyme whose presence would indicate maturity in oranges. Changes in the relative quantities of three enzymes in the fruit were studied, particularly during the ripening period. Of the three enzymes selected for study, only one (a phosphatase) seemed to show promise of meeting the requirements as a test to determine maturity of the fruit. Further research is needed to learn whether the decrease of this enzyme in measurable quantity actually coincides with full maturity of the fruit or whether the search must be continued.

Since certain organic sulfur compounds are known to undergo chemical changes activated by bacterial enzymes, with the formation of simpler sulfur compounds having disagreeable odors and flavors, a study of the sulfur compounds in citrus fruit was made, preliminary to investigating the bacterial enzymes that might decompose such compounds and thus contribute to off-flavor in citrus products.

Orange juice was found to contain recognizable quantities of a sulfur compound of the type being sought. A means for measuring the quantity of this compound and some preliminary steps for its isolation have been devised. A similar compound in asparagus was isolated and identified, and its constitution was proved by synthesis. This was done to establish certain chemical procedures to be used in the more complicated case of the citrus compound.

RIPENING OF CITRUS FRUITS STUDIED

During the 1947 citrus growing season a study was made of chemical changes that occur during the ripening of citrus fruits. Samples of oranges from the Winter Haven, Fla., laboratory and of grapefruit from the Weslaco, Tex., laboratory were sent to the headquarters of the Agricultural Chemical Research Division in New Orleans for examination by delicate chemical tests applied to thin sections of the fruit and observed microscopically. In each case samples of fruit from selected trees were taken at monthly intervals from the time the fruit was first set and less than one-half inch in diameter until it was fully mature. Sensitive tests with stains and color reactions for about 20 different classes of compounds were applied to microscopic sections at each stage in development and ripening of the fruits. These tests were qualitative, but the results showed that certain classes of chemical substances are present throughout development, while others may appear or disappear as the fruit develops and matures. For example, protein substance was detected in definitely localized deposits throughout development, but when the fruit ripened the protein became dispersed throughout the central portion of the juice sacs. Free tryptophan, an amino acid for which there is a sensitive and specific color test, was not found in the seeds of developing fruit but appeared at a high concentration in seeds of fully mature fruit. Since this test is easily applied to cut seeds, the appearance of this particular amino acid might serve as a test for maturity. During the 1948 growing season this study was being repeated under a Research and Marketing Act project to check previous results and to refine or perfect the methods of testing for those classes of substances whose appearance or disappearance appeared to be related to definite stages of fruit development.

WORK COMPLETED ON FEED YEAST FROM CITRUS-CANNERY WASTE LIQUORS

A large pilot plant for producing torula yeast from citrus-cannery waste liquors was operated again during the past season by the Agricultural Chemical Research Division's citrus products laboratory at Winter Haven, Fla., in cooperation with a commercial citrus cannery at Orlando, Fla. Operations were carried on continuously, 24 hours a day for 5 weeks. The quantity of waste liquors processed averaged about 180 gallons per hour. Fermentable-sugar content was somewhat lower than in previous seasons and averaged about 3½ percent. The yields of yeast under optimum conditions were 50 percent or more of the weight of fermentable sugar in the waste liquor processed. All of the operating data needed for the design of a full-scale plant and publication of a detailed report recommending operating procedures were obtained. The pilot-plant equipment has been dismantled and returned to the Southern Regional Research Laboratory.

Both the equipment and method of operation were considerably simplified this season as compared with the two previous seasons during which these experiments were carried on. Instead of having three yeast propagators in series, it was possible to operate with a single propagator, in which the growing time was about 2½ hours, at the same plant capacity and with as good yields as had been attained previously. This improvement was based on data which showed that little additional growth of yeast occurred in the second and third propagators. The use of a single propagator and shorter growing time greatly reduced the quantity of air required per pound of yeast produced.

The practicability of commercially processing unpasteurized citrus-waste liquor, which had been indicated in earlier experiments, was demonstrated during the 5 weeks of experimental operation on unpasteurized liquor. Some bacterial contamination developed during the last few days of the run, but this might be avoided, and reasonably long periods of large-scale operation maintained, by careful control of the operating conditions and the vigor of the culture. Aqueous ammonia was used as a source of nitrogen with as good results as were obtained with ammonium chloride or sulfate, or better, and at less cost. The necessary phosphorus was supplied economically in the form of trisodium phosphate. It was found that the waste liquor contained some utilizable nitrogen and phosphorus, which somewhat reduced the requirements for additional nutrients.

MORE ACONITATE RECOVERED FROM SUGARCANE MOLASSES

In its second season of full-scale commercial recovery of calcium aconitate from molasses by a process developed by the Agricultural Chemical Research Division, the largest sugar mill in Louisiana produced and marketed 360,000 pounds by this byproduct. This is more than 2½ times the amount produced during the previous season. There is an active demand for much larger quantities of this byproduct for conversion to aconitic acid, the esters of which are used in making transparent plastics of superior quality. The success of the operation is now well established, and the company is planning to process molasses from other sugar mills to extend the period of operation of its aconitate plant and greatly increase the output. In this way it expects to produce next season about 1,500,000 pounds of calcium aconitate from which about 1,000,000 pounds of aconitic acid can be obtained.

Research was continued at the New Orleans laboratory of the Division on the properties of the two different forms of aconitic acid and their salts to obtain basic data that might suggest means for recovering a higher percentage of the total aconitic acid in the molasses. Methods were developed for quantitatively separating the two forms of the acid, and the conditions under which the more soluble form is transformed into the less soluble form were determined. Also, additional information was obtained on the possibility of recovering aconitic acid from the leaves and tops of sugarcane and from immature cane of certain varieties that might be grown solely as a source of the acid. The latter possibility is being actively investigated by a sugar company with the cooperation of the Houma, La., laboratory of the Agricultural Chemical Research Division. Experimental plantings of prolific cane varieties known to have a high aconitic acid

content have been made for recovery experiments to be conducted this season.

DRY MILK PRODUCTS STUDIED AS CANDY INGREDIENTS

The Agricultural Chemical Research Division in cooperation with the National Confectioners' Association continued its research to extend the use of various agricultural food products in the manufacture of candy. Dry powdered milk products, which are available commercially, are especially desirable from the nutrition standpoint and can be easily shipped and stored. During the past year seven such products were incorporated in experimental batches of fondant, and their effects on quality and moisture retention were determined. Moisture retention is desirable for maintaining good texture and permitting longer storage of fondant candies. The commercially available milk products used were dry whole-milk solids, nonfat milk solids, whey powder, buttermilk solids, dried cream, lactalbumin, and casein. When 10 percent of any one of these materials was added to a standard fondant, it increased moisture retention of the fondant during the test period of 30 days. Fondant containing whole-milk solids, buttermilk solids, or dried cream lost only 50 percent of the initial moisture, whereas the plain fondant (without added milk product) lost 63 percent of its moisture under the same storage conditions. These ingredients not only impart desirable texture and flavor, but also increase the food value of the fondant.

TUNG OIL INDUSTRY HELPED BY RESEARCH

Research was continued by the Agricultural Chemical Research Division on the problem of drying and storing dehulled tung nuts for efficient handling at the oil mills. Studies of the moisture content of the nuts in equilibrium with surrounding air at various humidities yielded information that made it possible to recommend proper storage conditions for dehulled nuts dried to approximately 8 percent moisture at temperatures not over 175° F. When these conditions are maintained the nuts can be stored for 2 or 3 months without adverse effect upon the efficiency of oil extraction or upon the quality of oil obtained. The effects of storing raw tung oil under various conditions was also studied, because increasing quantities of the oil are being stored for fairly long periods of time. This work demonstrated that high temperatures should be avoided and that storage tanks should be nearly full. Raw tung oil stored in cans on the laboratory roof developed slight cloudiness. When cans were half filled and stored, there was a slight increase in viscosity and decrease in gelling time of the oil. The stored oil had not gelled (changed to the solid form) to any measurable extent.

In cooperation with the American Oil Chemists Society referee work was carried out during the past year to establish the accuracy of a method for determining oil in a ground sample of whole tung fruit. This method was proved to be rapid and practical in use by commercial analysts who are being called upon to make certified analyses of tung fruit for trading purposes. The procedure developed was recommended to the American Oil Chemists Society for adoption as a tentative method.

IMPROVEMENT IN GUM CLEANING BRINGS HIGHER RETURNS TO GUM FARMERS

During the year the Bureau's process for cleaning pine gum, and thus raising the grade of rosin produced, has been improved. More rapid filtration was made possible by determining the most suitable type of filter aid to add and establishing the best range of filtering pressures. Yields of rosin and turpentine were increased by recovering gum from the emulsion formed during washing and from the chips and trash removed in gum cleaning. Savings were also effected by cutting in half the proportion of oxalic acid used to remove iron stain from gum. These changes resulted in greater over-all efficiency of the central plants using this gum-cleaning process. The elimination of waste made it possible for these central plants to purchase gum on the basis of higher yields of turpentine and rosin, which resulted in correspondingly higher returns to the 40,000 farmers who supply the gum.

CONTINUOUS STEAM DISTILLATION OF PINE GUM IN PROSPECT

Processors of pine gum have closely followed the Bureau's work on continuous steam distillation of pine gum into turpentine and rosin and are awaiting final results from operation of the experimental stills to learn how they may profit by acquiring equipment for continuous steam distillation.

The 8-inch continuous column still described previously was operated to obtain data for use in developing one of larger throughput and improved design. In one series of tests, the open column was provided with baffle plates which made it possible to separate the last of the turpentine from the rosin with 25 percent less steam and to get a rosin of higher melting point. Although this saving of steam was offset by a 25-percent reduction in throughput, optimum baffling is advantageous. A 12-inch continuous column still was designed and built and is now being erected at the Naval Stores Station in Olustee, Fla. Engineering data previously obtained indicate that this larger unit will have more than twice the capacity of the 8-inch still. Operation of the 8-inch continuous still showed the possibility of a 50-percent saving in both steam and labor in comparison with the batch-processing steam still of the same capacity. Another advantage is that the turpentine tailings normally obtained during batch processing are absent in continuous distillation.

DIRECT ASSISTANCE GIVEN TO THE NAVAL STORES INDUSTRY

The technical staff of the Naval Stores Research Division continued to advise and assist producers and processors of pine gum on their immediate production and stilling problems, primarily to eliminate wastes, improve the yield and quality of their products, and increase the general efficiency of their plant operations. This help was rendered both at the station and in the field through personal contacts with plant owners. During the year 21 requests were received from processing plants for technical assistance; 24 conferences were held at the station with naval stores operators; 385 persons visited the station; and members of the station staff visited 40 gum-processing plants and 16 other gum naval stores operations. In addition, atten-

tion was given to 148 requests for general information, and 262 pieces of literature were distributed on request.

Gum-turpentine producers showed increased interest in the prevention of cloudiness and discoloration in turpentine during bulk storage and shipment. These faults were found to result in part from excessive amounts of gum or resin acids that are carried over with the turpentine vapor when stills are not properly operated. Close adherence to the distillation procedure recommended by the Bureau gives turpentine of better storage qualities.

FUNGUS AND ACID STIMULATION OF PINE-GUM YIELD RAISES QUESTIONS

Pine gum obtained from the Virginia pine (*Pinus virginiana*) by fungus stimulation was analyzed to learn whether it would yield good turpentine and rosin. This gum compared favorably with that obtained from slash and longleaf pines by normal chipping operations. It contained turpentine and rosin that were rather similar in composition to the gum spirits of turpentine and gum rosin of commerce. Fungus stimulation of southern slash and longleaf pines, which might reduce the cost of gum production, apparently would have no bad effects on the turpentine and rosin produced.

The use of acid for stimulating gum flow from pine trees introduces some acid into the gum and necessitates the use of acid-resistant receptacles for the gum. Therefore experiments were continued on evaluating various metals and alloys for use as turpentine cups for collecting such gum and coating materials for protecting ordinary metal cups. The tests were made on conventional and experimental materials including aluminum and aluminum alloys, Monel metal, nickel, stainless steel, electrolytically galvanized sheet iron and painted sheet iron, together with vitreous-enamelled steel. The most satisfactory results were obtained with the enamelled steel and two aluminum alloys. Some of the materials broke down promptly. Both the positive and negative information obtained is of importance to the naval stores industry as a guide in the selection of cup materials.

TERPENE HYDROCARBONS YIELD NEW CHEMICALS

The peroxide-catalyzed reaction that yields addition compounds of organic chlorides with various terpene hydrocarbons obtainable from turpentine was found to be applicable to the preparation of addition compounds of terpenes with silicon-containing organic chlorides. The presence of the silicon-hydrogen (Si-H) linkage in the silicon compounds appears to be necessary. Thus, silicon tetrachloride ($SiCl_4$), unlike carbon tetrachloride (CCl_4), did not undergo this type of reaction, but trichlorosilane ($SiHCl_3$) and methyltrichlorosilane (CH_3SiHCl_3) were successfully added to such representative terpenes as alpha-pinene, beta-pinene, dipentene, and camphene. The silicon-containing addition compounds impart water-repellency to surfaces of fabrics, glass, and vitreous enamel. By polymerization they can be converted to elastic substances.

When the turpentine derivative allo-ocimene is heated, a complex mixture of hydrocarbons is obtained. The major constituent of the mixture was identified and found to have a conjugated cyclohexadiene structure. Since such compounds are very reactive chemically, the

new product can be used for various reactions of interest in organic synthesis. In the course of this work, several new isomeric cyclohexadienes were isolated. The presence of some aromatic hydrocarbons in the mixture was also established.

RUBBER CHEMICALS AND VARNISHES MADE FROM PINE-GUM DERIVATIVES

Work was continued on the use of the sodium salt of the partially esterified addition compound of levopimaric acid and maleic anhydride as an emulsifying agent in the preparation of synthetic rubber. Butadiene-styrene copolymers formed in the presence of this emulsifying agent showed excellent milling characteristics and good tack and, when suitably compounded, had tensile strengths of from 4,100 to 4,400 pounds per square inch of cross section with elongations of from 500 to 700 percent at the break.

A procedure was developed for the preparation of good water-resistant varnishes from linseed oil and the maleic anhyride addition product of levopimaric acid obtained from pine gum. A mixture of the mono-, di-, and tri-glycerides of linseed-oil acids was prepared by heating linseed oil with glycerol. The free hydroxyl groups introduced by this reaction were then esterified by further reaction with the maleic-levopimaric addition compound. The high-molecular-weight esters thus formed were diluted with turpentine or mineral spirits to form spirit varnishes. These varnishes exhibited desirable characteristics. Air-dried films were impervious to water for 72 hours at room temperature. After being oven-baked, the films showed no visible cloudiness even after 20 to 30 days' immersion in water at room temperature. After being immersed 18 to 20 hours in boiling water, the oven-baked films frequently peeled off the test panels but still did not show any cloudiness. Although the air-dried and oven-baked films showed excellent water resistance and desirable hardness, all of these varnishes have some objectionable features. Thus, the reaction conditions seem to be quite critical, making it difficult to duplicate a particular varnish. The long periods of time required for esterification and the high acid numbers of the varnishes are other disadvantages. In spite of these drawbacks, results are sufficiently promising to warrant further investigations.

A hitherto unreported chemical compound was found in both rosin and pine gum. While its complete structure has not been definitely established, enough has been learned about the molecular arrangement and chemical reactions of this compound to prove that it is new. The compound has a phenanthrene ring structure with a double bond in the 7, 8 position conjugated to a double bond in a side chain.

PILOT-PLANT STUDIES BEGUN ON PRODUCTION OF NEW PINE-GUM DERIVATIVE

A project on the pilot-plant production of an addition compound (adduct) of maleic anhydride with the levopimaric acid in crude pine gum was begun in November 1947 under provisions of the Research and Marketing Act of 1946. A laboratory process mentioned in previous reports and patented by the Bureau was the starting point for the pilot-plant work. By the end of the fiscal year a pilot-plant line for processing 100-pound batches of crude gum had been con-

structed and was in operation. It was equipped to produce turpentine and a maleic rosin in addition to the maleic-levopimaric acid adduct, but at the start work was concentrated on the preparation of the adduct.

This small-scale equipment gives an adduct of high quality, apparently suitable for commercial use without further processing. A representative sample of the crystalline compound had a melting point of 428°–435° F., an acid number of 251, and a saponification number 354. Adducts of identical quality were produced by bringing about the reaction in either turpentine or petroleum-naphtha solution. However, use of the naphtha presents a separation problem in the turpentine-recovery step. On the other hand, the naphtha permits better crystallization than does turpentine, unless absolutely fresh turpentine is used. Substantial samples of the adduct were supplied to industrial chemical firms, including manufactures of paint, varnish, and lacquer, for experimental use and tentative evaluation. Preliminary reports were favorable.

Plans are in progress for increasing the capacity of the pilot-plant so that 1 barrel (435 pounds) of crude gum can be processed at one time. The larger production line will facilitate determination of commercial operating procedures and production costs.

PLANTS SURVEYED AS SOURCES OF ANTIBIOTICS

The need for additional antibiotics for combatting infectious diseases has resulted in the establishment of a new project under the Research and Marketing Act of 1946 on the production of antibiotics from agricultural sources. The Bureau's Biologically Active Compounds Division at Beltsville, Md., has already started work under this project on the isolation of antibiotic substances from plants.

The extracts from 34 plants have been assayed for antibiotic activity. Water and methyl alcohol were used to extract soluble substances from the prepared plant materials. In general, methyl alcohol extracted more antibiotic substances than did water. In tests of antibiotic activity against typical micro-organisms, 12 of the extracts showed antifungal activity against *Fusarium oxysporum* f. *lycopersici;* 12 inhibited the growth of the Gram-negative organism *Escherichia coli;* 19 inhibited the growth of the Gram-positive organism *Staphylococcus aureus;* and some inhibited the growth of the acid-fast organism *Mycobacterium phlei.* Some of the more promising plant extracts were also tested against other micro-organisms. Investigations on the isolation and characterization of the active antibiotic principles in some of the promising plant extracts are in progress.

COTTONSEED PIGMENT GLANDS STUDIED AS A SOURCE OF ANTIBIOTICS

Under the new Research and Marketing Act project on the production of antibiotics the pigment glands of cottonseed, which are separated from the flaked kernels by a new process developed at the Southern Regional Research Laboratory, were investigated to determine whether they contain antibiotic substances. These pigment glands comprise 3 to 4 percent of the cottonseed meats.

Gossypol, one of the pigments of cottonseed, was tested by the Bureau's Biologically Active Compounds Division and found to have

antibacterial activity but little or no antifungal activity. Furthermore, gossypol inhibited the growth of Gram-positive bacteria but did not inhibit the growth of the Gram-negative bacteria tested. The following micro-organisms were inhibited by gossypol: *Bacillus subtilis*, *Bacillus mycoides*, *Mycobacterium phlei*, *Staphylococcus aureus*, and *Streptococcus lactis*. Gossypol does not appear to lose its antibacterial activity when treated with an excess of sodium hydroxide or hydrochloric acid. Its activity diminishes, however, with addition of ferric chloride to the basal medium.

Tests for antibiotic substances other than gossypol in pigment glands from cottonseed were made by incorporating the ground pigment glands directly in the nutrient media for the organisms, but only inhibitory activity toward Gram-positive bacteria was detected. Then the pigment glands were fractionated into three portions, as follows: (1) They were exhaustively extracted with hot ethyl alcohol, and the alcohol extract was evaporated to dryness. A water extract of the alcohol-soluble substances showed no inhibitory activity toward any organism; an extract prepared with a weak solution of alkali and then neutralized showed antibacterial activity equal to that of gossypol. (2) The alcohol-extracted pigment glands were then exhaustively extracted with hot water, and the water extract was evaporated to dryness. This extract showed no antibiotic activity. (3) The residue from alcohol and water extraction of the pigment glands constituted the third fraction. When it was incorporated directly in the nutrient media for the test organisms, no antibiotic activity was observed.

It would appear that most of the antibiotic activity of the pigment glands is due to gossypol or closely related substances and that the pigment glands contain no antifungal substances.

TOMATINE AND TOMATIDINE ISOLATED FROM THE TOMATO PLANT

It was stated in the 1947 report that "pure tomatin" has been isolated in crystal form from extracts of the tomato plant by the Biologically Active Compounds Division. During the past year crystalline tomatin was characterized as a glycosidal alkaloid having a molecular weight of approximately 1050. Therefore, in conformity with the practice in naming alkaloids, the name was changed to "tomatine." Tomatine, when hydrolyzed with acid, splits into two major fractions; one is the basic alkaloid, which has been named tomatidine, and the other is essentially a mixture of sugars. The tomatidine is readily crystallized and has a molecular weight of approximately 400.

Since tomatine is an alkaloid it seemed possible that it might be identical with solanine, the alkaloid from the potato plant, which belongs to the same plant family. However, biological, chemical, and physical measurements showed that tomatine and solanine are two distinct compounds. It was learned that the tomatidine portion of tomatine is responsible for the antifungal activity of the latter. This is in marked contrast to the activity shown by degradation products of other antibiotics, since hydrolysis of antibiotics usually decreases or completely destroys their activity. It seems essential that the clinical effectiveness of tomatidine, as well as that of tomatine, be evaluated.

Tomatine was produced on a pilot-plant scale with facilities of the Eastern Regional Research Laboratory. Two methods of extraction

were used, including: (1) Extraction of dried tomato leaves with alcohol; and (2) extraction of dried tomato leaves with dilute hydrochloric acid solution. Either ethyl or methyl alcohol can be used in the alcohol-extraction process. The alcohol extract is evaporated to a small volume, and most of the alcohol is recovered by condensation. The alcohol concentrate is diluted with hot water and stirred, and the acidity of the liquid is adjusted to about pH 5.5. Under these conditions the tomatine dissolves and the fats and lipids settle to the bottom of the tank. Extraction of the dried leaves with dilute hydrochloric acid solution (pH 5.5) is advantageous because no organic solvent is required, the amount of fat or lipid material extracted is negligible, and the extract is readily filtered. Either the clarified acid extract of the tomato leaves or the acid-soluble portion of the alcohol extract is then adjusted to pH 10.0, whereupon crude tomatine is precipitated. The precipitate can be collected by centrifugation. Crystallized tomatine can be prepared by dissolving the crude tomatine in alcohol or dioxane and allowing the solution to evaporate.

Tomatidine, the nonsugar fraction of tomatine that retains the antifungal properties, is readily obtained by acid hydrolysis of crystalline or crude tomatine. Tomatidine is first isolated in the form of an acid salt which is converted to the free base by adding ammonia to the solution. The relative clinical effectiveness of tomatine and tomatidine may, to a considerable extent, determine the commercial interest in the latter. The Bureau can act in an advisory capacity on methods for commercial production.

The effectiveness of tomatine in protecting tomato plants from fusarium wilt is being investigated in cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering. Since tomatidine was found to possess the antifungal properties of tomatine, some of the earlier work on this plant disease must be reevaluated in the light of this finding. It is significant that tomatine has been isolated from the Red Currant tomato plant (*Lycopersicon pimpinellifolium*), which variety is considered to be immune to fusarium wilt. Investigations are in progress to determine if this same substance occurs in tomato plants of other species and varieties. Variation in the tomatine-like substance in different species and varieties of tomato might be an important factor in explaining the variation in disease resistance of tomato plants.

RUTIN REDUCES ANTIFUNGAL ACTIVITY OF TOMATINE

Rutin is recognized as one of the most widely distributed plant glycosides (sugar complexes), although it is produced commercially only from buckwheat plants. Incidental to the research on tomatine, crystalline rutin was isolated from extracts of the Red Currant tomato plant (*Lycopersicon pimpinellifolium*) by the Biologically Active Compounds Division. The chromatographic technique was used to fractionate the extract. When the crude tomato-plant extract was passed through a chromatographic column of potato starch, rutin crystallized from the first part of the filtrate. Following the separation of rutin crystals, the supernatant solution was tested against micro-organisms and found to have very high antifungal activity. This indicated the presence of tomatine. Later fractions taken from the column showed less antibiotic activity, and the rutin could not be

isolated. These results indicated that the antifungal principle (tomatine) in the tomato-plant extract might be a glycoside like rutin because compounds that pass through a chromatographic column at similar rates would be expected to be of similar chemical nature.

When crude tomato-plant extract was incorporated in the culture medium it failed to inhibit the growth of two strains of the fungus *Candida albicans*, whereas crystalline tomatine completely inhibited them. Since rutin was found to be a normal constituent of the crude tomato-plant extract, the effect of rutin and its nonsugar fraction, quercetin, on the antibiotic activity of crystalline tomatine toward *Candida albicans* was investigated to learn whether either of these compounds could account for the observed difference in the antibiotic properties of crude tomato-plant extracts and crystalline tomatine. In the presence of rutin or of quercetin the fungistatic activity of crystalline tomatine toward *Candida albicans* was markedly reduced or completely neutralized, depending upon the concentration of the particular ingredient.

These results are significant because they fit in with observations previously made at the Eastern Regional Laboratory. It was found there that rutin did not have any antibiotic activity toward bacteria or fungi. Quercetin exhibited considerable antibiotic activity toward Gram-positive and certain Gram-negative bacteria, but had no effect on the growth of certain fungi. Neither rutin nor quercetin was toxic to the fungus *Candida albicans*. It appears now that the antibacterial activity observed in crude tomato-plant extracts may be accounted for, in part, by the presence of small amounts of quercetin, although probably other substances were also present.

The biochemical reasons for disease resistance in plants, particularly in the tomato plant, are undoubtedly complex, but this Bureau has now established that the Red Currant tomato plant, which is immune to fusarium wilt disease, contains both an antifungal substance, tomatine, and an antibacterial substance, quercetin. Quercetin may be combined with a sugar to form the glycoside rutin, and both rutin and quercetin, if present in sufficient quantity, are capable of neutralizing the antifungal activity of tomatine toward certain organisms. In cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering, tomatinelike substances will be isolated from tomato varieties of varying degrees of wilt resistance for further research on their composition and antibiotic properties. Tomatine has been shown to be a good antifungal agent in laboratory cultures. If it should prove to be of medicinal value in combating fungus diseases of animals, it would be important to see than rutin and quercetin are not administered at the same time.

NEW ANTIBIOTIC, POLYMYXIN, MAY BECOME IMPORTANT DRUG

The extraordinary development of penicillin as a new kind of weapon against disease has naturally stimulated a search for other antibiotic substances. Discovery of at least a hundred has been claimed, but only a limited number offer promise in the medical field. Toxicity, lack of solubility in body fluids, and progressive loss of effectiveness against virulent organisms are some of the difficulties encountered. One of the more promising antibiotics is "polymyxin," which is produced by *Bacillus polymyxa*. Its discovery was reported first by the

Northern Regional Research Laboratory in May 1947 and subsequently by research laboratories of a chemical manufacturing company in this country and a pharmaceutical manufacturing company in England.

By 1945 the outstanding contributions of the Northern Regional Laboratory to the development of penicillin had been successfully applied in commercial practice. After completion of the research program on penicillin, microbiological research was directed toward other fields. Since penicillin inhibited only Gram-positive organisms, there was a need for antibiotics that would inhibit Gram-negative organisms, particularly those causing *Brucellosis* in farm animals and undulant fever in man. Therefore exploration was made in this direction. Attention was also paid to the possibility of turning up an agent that would be effective against tuberculosis. A great many soil bacteria were investigated by means of plate screening tests, and several strains of *B. polymyxa* were selected for study. Curiously, it is this same organism which was used during the war to produce butylene glycol from grain in the development of new sources of butadiene for synthetic rubber manufacture.

The combined efforts of the Northern Laboratory's investigators and of other research workers have revealed several properties of polymyxin. It is a polypeptide and is basic, forming salts such as the hydrochloride. It is quite stable either in water solution at low pH or as a dry substance. It can be purified so that 1 or even ½ microgram per milliliter will inhibit growth of the test organism. The Laboratory's recent contributions include an improved assay procedure, a more productive organism obtained through mutation induced by ultraviolet irradiation, and a better method of feeding the organism. The yield of the antibiotic is several times what it was previously. Some evidence was obtained that a second antibiotic factor is produced by *B. polymyxa*, and the possible existence of a whole family of polymyxins, as is the case with penicillins, must be recognized.

It was originally found that polymyxin would inhibit the growth in laboratory cultures of representatives of eight different groups of Gram-negative organisms, including the infective agents of brucellosis and paratyphoid A and B. These results suggested the possibility of polymyxin being effective against the etiological factors in Asiatic cholera, bacterial dysentery, tularemia, plague, and additional *Salmonella* infections. As a matter of fact, other investigators have shown that fairly low concentrations of the antibiotic sharply inhibit most of these organisms as well as a representative of the pneumonia family and members of the *Hemophilus* group, including the agent of whooping cough.

Before any antibiotic is finally accepted for medicinal use, it must, of course, be proved to be safe and beneficial by tests on animals and voluntary experimental use by humans. Arrangements were made with the National Institute of Health to test the efficacy of polymyxin in treatment of animals infected with various diseases, and one extensive trial has been made. Guinea pigs were heavily inoculated with *Brucella suis* and two weeks later were treated with polymyxin up to 9 milligrams a day. There was little, if any, apparent benefit. However, since this organism was inhibited by polymyxin

in laboratory cultures, it seems possible that it might be sharply inhibited by larger doses in animals. It is intended to extend this work.

Other investigators have found polymyxin (or aerosporin) to be effective in mice against *S. typhi*, *H. pertussis* (whooping cough), *H. influenzae*, *Pasteurella multocida*, *Klebsiella pneumoniae*, and other organisms. Furthermore, it has been found both here and abroad that polymyxin exerts a marked ameliorative effect on the course of whooping cough in humans. It has also been reported in this country that two out of four cases of undulant fever were successfully treated with polymyxin. Infant diarrhea, among other infections, appears susceptible to treatment with this antibiotic. These experiments, of course, will need to be repeated and extended, but it appears that polymyxin will find definite application in medical practice.

RESEARCH ON ALLERGENS YIELDS IMPORTANT RESULTS

Fundamental research on the chemical nature, distribution, and physiological action of allergens with respect to their influence on the utility of agricultural products was continued by the Allergen Research Division under a project supported by an allotment from the special research fund authorized by the Bankhead-Jones Act of June 29, 1935. During the year further attention was given to the allergens of oilseeds and nuts. Work was also done on incidental problems including the development of a reliable quantitative method for determining tryptophan in allergenic proteins and the acquisition of additional data on the factors that influence the anaphylactic response of sensitized laboratory animals to antigens, in the hope of making the test quantitative for the potency of allergens and allergenic fractions.

ALLERGENIC FRACTIONS ISOLATED FROM FLAXSEED AND NUTS

More than twenty-five-fold increase in yield of the allergenic fraction, FS–1A, from flaxseed resulted from a preliminary treatment of the seed to assure virtually complete elimination of the flaxseed hulls. A mucilage-like component of the hulls interfered with fractionation of the water-soluble proteins and caused excessive losses of the proteose fraction FS–1A. A newly devised procedure prevents these losses. Most of the hull material can be separated by grinding and sieving the thoroughly defatted seeds. To secure a hull-free and fat-free flaxseed meal, a final separation was accomplished by flotation in a chloroform-petroleum ether medium from which the hull particles precipitated during centrifugation. Hull-free meal separated as a floating layer which was easily recovered.

From this meal the yield of the allergenic fraction FS–1A was 1.98 percent, while the best previous yield without complete removal of the hulls was 0.07 percent. The present yield of the 1A (allergenic) fraction from flaxseed is higher than has been obtained from any other oilseed; the nearest were castor bean and cottonseed, which yielded 1.76 and 1.14 percent, respectively.

Other oilseeds from which the allergenic proteose fraction was isolated during the past year were black walnut, almond, and Brazil nut. Yields of the 1A fraction from these ranged from 0.08 to 1.98 percent on weight of fat-free meats. The nitrogen content of these new allergens falls in the same range as those previously studied.

ALLERGENS FROM TWO VARIETIES OF FILBERT HAVE SAME PROPERTIES

Allergenic fractions previously isolated from two distinct varieties of filbert nuts provided suitable material to determine whether immunologic properties reflected varietal differences in oilseeds. Filbert 1B fractions—essentially similar to the 1A fractions of other oilseeds—derived from Barcelona and DuChilly filberts were precisely alike in their capacity to sensitize guinea pigs. These allergenic fractions from filberts resembled the corresponding fractions from cottonseed and castor beans in their stability to heat (being unaltered in potency or specificity when heated for 1 hour at 95° to 100° C.) and in their comparatively small molecular size (implied by their rapid passage through a dialyzing membrane that retains most native proteins).

MORE EXACT ANALYSIS OF ALLERGENIC AND OTHER PROTEINS MADE POSSIBLE

Correlation of chemical constitution with immunologic properties of an allergen requires knowledge of the kinds and proportions of the amino acids it contains. Development of a better method for the determination of tryptophan, an important amino acid, has been one of the objectives of the work on the chemical characterization of allergens for more than 2 years. The usual methods for determining this amino acid proved unsuitable for the allergenic proteins because significant losses of tryptophan result from the alkaline hydrolysis that is required at the beginning of the analysis. Studies of factors that affect critical color-forming reactions of tryptophan were completed this year and led to the recognition of new principles on which an improved, more accurate method for determination of tryptophan was based. Advantages of the new method include: adaptability to direct analysis of proteins in solid state or in solution; reliability and high precision with samples of only a few milligrams in weight; suitability for samples containing carbohydrate; and assurance that the protein is not altered by a preliminary hydrolysis. The method was tested on representative samples of various proteins in their native and separated states and found to be reliable.

PROGRESS MADE TOWARD MEASUREMENT OF ANAPHYLACTIC REACTIONS TO ALLERGENS

Investigation of the various controllable conditions that may influence the reactions of guinea pigs to allergens was directed toward learning the effects of adjuvants accompanying the sensitizing agent. During the past year studies were completed on the effect of treating the antigen with alum and the effect of the route of administration of the antigen on the size of dose required to induce anaphylactic sensitization. The alum-treated antigen, ovalbumin, had a sensitizing capacity of 4 to nearly 400 times that of the untreated control. This wide range was related to the route of administration. For the control, ovalbumin in saline, the sensitizing capacity of the antigen by intra-abdominal administration was about one and one-half times greater than by vein, and nearly two and one-half times greater than by subcutaneous injection. For alum-precipitated ovalbumin, the sensitizing capacity by the subcutaneous route was 2½ times greater than by intra-abdominal administration and 60 times greater than by intravenous injection.

These results reveal the wide variations in the anaphylactic sensitivity of guinea pigs that is induced by different dosage levels of antigen, with and without adjuvant. Route of administration of the antigen is also shown to influence in large measure the acquired sensitivity. While the anaphylactic reaction has long served for demonstrating the antigenic character of proteins, little attention has been given to this reaction for quantitative measurements of potency. Here for the first time the important influence of an adjuvant and the method of sensitizing the animal are revealed in quantitative terms. These fundamental studies are being continued to determine the interrelationships of these factors over a considerable period of the animal's life span.

FURTHER PROGRESS MADE IN RESEARCH ON ALLERGENS OF JOHNIN AND TUBERCULIN

Johnin and tuberculin are sterile bacterial products prepared from cultures of Johne's bacillus and tubercle bacillus for differential diagnosis of Johne's disease and tuberculosis in cattle. Chemical and immunological investigations of johnin and tuberculin were begun last year by the Allergen Research Division in cooperation with the Bureau of Animal Industry to distinguish allergens or antigens in these diagnostic agents that are responsible for skin reactions induced by latent infection with Johne's disease or tuberculosis of cattle when the agents are injected into the skin. In the early stages of the diseases, either johnin or tuberculin occasionally produces a false positive skin reaction, leading to incorrect diagnosis or uncertainty. It is believed that knowledge concerning antigens common to both johnin and tuberculin and those characteristic of each will make possible increased accuracy of disease diagnosis in livestock.

Immunologic studies of the first year showed that the anaphylactic sensitization of guinea pigs with johnin and tuberculin provided a feasible laboratory procedure for detecting and measuring the effect of fractionation on antigenic potency and specificity. Laboratory tests with guinea pigs also proved that johnin and tuberculin contain antigenic components that are common to both products. The presence of antigenic components that are different and characteristic of each product was also demonstrated by the reactions of sensitized guinea pigs. Proof of the presence of common antigens in tuberculin and johnin corresponds with the observation that skin tests on cows with these products lack sufficient specificity to provide completely reliable diagnostic evidence for identifying and differentiating Johne's disease and tuberculosis.

TUBERCULIN AND JOHNIN FRACTIONATED

Demonstration of the presence of distinguishably different antigens in tuberculin and johnin implies the possibility of isolating the characteristic antigens of each product by fractionation. Accordingly trial of various chemical and physical fractionation procedures was continued during the past year. The procedures tried included: (1) fractional precipitation of the protein components with lead acetate, picric acid, alcohol, and hydrochloric acid; and (2) fractional concentration by physical methods such as dialysis, ultra-filtration, and adsorption.

The most promising fractionation procedure was precipitation of the protein components of tuberculin or johnin with picric acid or hydrochloric acid. Fractionation of tuberculin by precipitation with picric acid at various pH levels was studied to determine whether potency and specificity could be increased by this method. Eighteen series of fractionations were made and evaluated for changes in potency by comparison with the potency of a particular fraction, T–31, which was adopted arbitrarily as a tentative standard of reference. Fraction T–31 was the dried precipitate obtained by treatment of regular tuberculin with 90-percent alcohol. Potency tests of two series of fractionation products showed wide variations among potency values obtained in individual skin tests with the same products. These variations indicated the influence of some unrecognized factors of the skin reaction and necessitated statistical evaluation of many repeated tests. However, the results provided evidence of increased potency which was favorable for two fractionation procedures. For example, a picrate fraction (T–37–4) showed an increase of 6.2 times in potency of the homologous (tuberculin) antigen and a decreased potency of 0.5 in the heterologous (johnin) antigen. Thus, in this fractionation there was obtained evidence of substantial increase in potency of the principal antigen and a decrease in the activity of the johnin antigen.

These observations require further correlation with chemical evidence that protein components were concentrated and with results of diagnostic skin tests on cows. A potentially important advance was made this year in the fractionation of unconcentrated tuberculin by precipitation of its protein components with hydrochloric acid. For example, addition of hydrochloric acid to 7 liters of unconcentrated tuberculin, to pH 5, yielded 6.5 grams of precipitate containing 2.8 percent of ash and 15.7 percent of nitrogen. When this precipitation procedure was applied to tuberculin that had been concentrated, without heating, from 7 liters to 0.7 liter, the yield of the precipitate was 3.9 grams. Upon reprecipitating this fraction from a 1-percent aqueous solution by adding hydrochloric acid to pH 5, 1.9 grams of a fraction containing 1.8 percent ash and 15.8 percent nitrogen was obtained. Fraction T–31, adopted as the standard of reference, contained 4.8 percent ash and 10.9 percent nitrogen. Thus, precipitation of tuberculin from the culture fluid with hydrochloric acid promotes the separation of fractions that contain comparatively less ash and more nitrogen. This trend of changing chemical composition justifies the expectation of an important advance in purification of the biologically active components of tuberculin, with a corresponding improvement in diagnostic value.

Current progress in advancing the potency and specificity of tuberculin by fractionation is limited by the rate of progress that can be made in immunologic evaluation of the products of fractionation. Chemical analysis provides evidence of collateral value to immunologic data. However, for guidance of fractionation procedures, the data from immunologic assay of potency and specificity are indispensable. Results of skin tests on cows with fractionation products of both tuberculin and johnin need to be correlated with data obtained by evaluation of potency and specificity of the anaphylactic antigens of the same products from tests on guinea pigs. At present, disparity between the results obtained in tests on guinea pigs and those obtained

in tests on sensitized cows necessitates further study to determine whether the results of these two methods can be correlated. The possibility is recognized that the two immunologic reactions are induced by unrelated antigens.

RELATIONSHIP OF JOHNIN AND TUBERCULIN ANTIGENS STUDIED

In addition to studies of chemical fractions of johnin and tuberculin, experiments were conducted during the past year to determine the antigenic relationship of these two bacterial products. Methods employed in these studies have been based on the anaphylactic reactivity of excised smooth uterine muscles from guinea pigs that have been sensitized by injection of either johnin or tuberculin. This procedure departs from the usual method of measuring the potency and specificity of these products by direct skin tests on sensitized cows. The objective of this phase of the study of johnin and tuberculin is to devise a cheaper, more rapid, and more flexible procedure than the direct skin test for guidance of chemical fractionation of johnin and tuberculin.

Other workers in this field have proposed the following hypotheses to explain the cross reactions of johnin and tuberculin: (1) Johnin and tuberculin each contains, in addition to its specific antigen, a certain proportion of one or more antigens characteristic of the acid-fast group of bacteria; and (2) johnin and tuberculin each contains identical or closely similar antigens that differ only in the quantitative relationship of the antigens in each preparation.

Experiments designed to determine which of these two hypotheses is correct have not yielded clear-cut results. The observation was previously reported that when guinea pigs were sensitized with tuberculin the uterine muscles from these animals were about eight times more sensitive to tuberculin than to johnin. Likewise, when the animals were sensitized with johnin the excised uterine muscles were about six times more sensitive to johnin than to tuberculin. Moreover, when the uterine muscles from sensitized animals were first desensitized with the heterologous antigen a subsequent reaction could be obtained with a second addition of an equivalent amount of homologous antigen. On the other hand, when the tissues were first desentized with homologous antigen, subsequent addition of the heterologous antigen did not induce further reactions. These observations appeared to confirm the first hypothesis.

Later experiments have cast some doubt on this interpretation. For example, if a guinea pig is sensitized with tuberculin and the uterine muscle is mounted in the Dale bath one unit of tuberculin (homologous) will desensitize only to about six units of johnin (heterologous). Moreover, as little as four units of johnin will desensitize to one unit of tuberculin. These ratios of desensitizing doses do not seem to be large enough to offer confirmation of the first hypothesis. In similar tests on johnin-sensitized animals 1 unit of johnin (homologous) desensitized to nearly 100 units of tuberculin (heterologous), and 25 units of tuberculin desensitized to 1 unit of johnin.

It is clear from these experiments that, if the first hypothesis is correct, johnin contains a greater concentration of the common antigen than tuberculin. But if each product contains an additional specific antigen no amount of the heterologous antigen should com-

pletely desensitize to the homologous antigen. This interpretation, however, does not take into account the possibility of nonspecific desensitization.

Convincing evidence of the existence of a specific antigen in johnin is seen occasionally in guinea pigs that are presumed to be sensitized to the whole complex of antigens in johnin. For some reason, not yet determined, a few of these animals show very strong sensitivity to the homologous antigen and very little, if any, sensitivity to the heterologous antigen. This type of sensitivity has appeared irregularly and will require further study to define the conditions on which it depends. The most important immediate objective is to identify a specific skin-reactive antigen in johnin.

REACTION OF PROTEINS WITH FORMALDEHYDE CLARIFIED

The use of formaldehyde to stabilize protein materials in industrial applications, as in tanning of leather, hardening of casein plastics, and waterproofing of protein adhesives and coatings, is well known. But, heretofore, the mechanics of the reaction of protein and formaldehyde has not been thoroughly understood, although there have been indications that this reaction varies with different classes of proteins—for example, casein and zein.

In a series of detailed studies at the Western Regional Research Laboratory with model substances (compounds resembling proteins in molecular size and structure but having a preponderance of only one group) it was found that formaldehyde supplies methylene groups which link the amino groups on the one hand with amide, guanidyl, phenol, imidazole and indole groups on the other. Thus a protein composed of a relatively large amount of lysine (straight amino acid) and average amounts of arginine, tyrosine, and tryptophan (amino acids containing guanidyl, phenol, and indole groups, respectively), when reacted with formaldehyde forms crosslinks to produce a more stable and more water-resistant product. If such a protein is spun into fibers which are subsequently treated with formaldehyde the physical properties of the fibers will be improved by the formaldehyde reaction.

Now, with the mechanics of the reaction known, it is possible to build new protein derivatives by linking the amino groups of protein with the desired low-molecular-weight amine, amide, guanidine, and other compounds by means of formaldehyde. By this means some antibiotic derivatives having desirable "built-in" properties have been prepared. These indicate the possible practical application of results of this fundamental study of the protein-formaldehyde reaction in fields other than tanning or hardening of proteins.

CERTAIN GROUPS IN PROTEINS RESPONSIBLE FOR WATER ABSORPTION

A study of the groups responsible for uptake of water vapor in proteins has been carried out with success in the Eastern Regional Research Laboratory. The warm feel of fabrics made of protein fibers, both natural and synthetic, is related to this property. Investigation has shown that the free amino groups of casein account for one-fourth of the water absorption and the peptide backbone of the protein accounts for about one-half of the total. In silk, where there are very

few reactive side groups, over 95 percent of the total water is absorbed by the backbone. These facts, together with basic information about the amino acid composition of the proteins, make it possible to predict the amount of water a protein will take up and the effects of the various chemical reactions used in making protein fibers and plastics on this important property.

WHEAT GLUTEN AND FAT FOUND TO COMBINE WHEN FLOUR IS WET

Wheat gluten obtained simply by washing starch and other components out of flour dough contains (besides proteins) starch and also some fat (up to 10 percent) that cannot be extracted by fat-solvents. It was discovered at the Western Regional Research Laboratory that, although most of the fat can be extracted from dry flour with organic solvents, a considerable part becomes resistant to such extraction when flour is wet with water, and still more so when the flour is kneaded into dough. When the starch was washed out of flour, the fat remained with the gluten. Ability to form a complex with fat during wetting and doughing may be unique for the proteins of wheat since this property has not been observed with other proteins. Further fractionation showed that the bound fat stayed with the glutenin rather than the gliadin fraction of the protein.

Since the bread-making quality of flours is related to proportion and properties of the gluten, it was considered important to determine if this tendency of wheat gluten to "bind" fat is correlated with baking properties of the flour. A number of wheat flours of known baking behavior are being examined with reference to their fat-binding capacity.

Efforts to determine other chemical characteristics responsible for baking behavior are continuing. During the course of this work it was found that flour doughs and gluten have the capacity to bind considerably more fat than is normally present in flour. The possibility of making new types of protein derivatives by this technique remains to be determined.

STRUCTURE OF HIDE PROTEIN SHOWN BY ELECTRON MICROSCOPE

The hide-and-skin substance principally involved in leathermaking is the protein collagen. It is composed of fibrils too small to be seen except with an electron microscope. These fibrils are transversely striated, with a normal interval of 660 Angstrom units (A). At the Eastern Regional Laboratory it was found that several tannages, including formaldehyde and canaigre, reduced the interval to as little as 550 A. The regular collagen structure appeared to have contracted some 15 per cent without noticeable distortion. At pH 3 or lower, common acids such as acetic and hydrochloric reduced the periodicity by a comparable amount, and also split the fibrils lengthwise into a number of threads of even smaller diameter.

A sharkskin collagen was distinguished from the collagen of higher animals in that even in water the fibrils demonstrated very extensive lengthwise cleavability. Width of the cleavage fragments was as little as 100 A, and the threads were too thin to be striated. An explanation of the difference in fibril properties among collagens is being

sought both in the chemical makeup of the collagen and in substances that are thought to cement the fibril elements together.

When heated in water, collagen and leather shrink markedly within a small temperature interval. The shrinkage temperature varies with the animal species, and is raised by tanning and depressed by swelling agents. In the electron microscope cowhide-collagen fibrils heated at 63° C., five degrees below the ordinary shrinkage temperatures, showed pronounced local swelling. The swelling occurred most frequently at fibril ends, but was found also at random positions along the fibrils. When there was no pronounced swelling, the fibrils were striated. Above 75° C. all fibrils were swollen completely and were distinguished by their relatively short length, compared with unheated fibrils. The appearance of a swollen fibril was that of a thread in the form of a loose, flattened spiral, embedded in a transparent film. As the temperature was raised, the spiral core became less prominent and the film became opaque. The reaction appeared to be one of progressive, irreversible gelatinization of the collagen. Formaldehyde tannage, which raised the shrinkage temperature to 88°, correspondingly raised the temperature at which fibril disintegration set in.

STRUCTURES OF STARCH COMPOUNDS SHOWN BY X-RAYS

The physical and, to some extent, the chemical properties of starch are determined by the structural configurations which the constituent glucose chains of starch can assume under various conditions. Determination of the structure of amylose, one of the principal components of starch, and its compounds by the X-ray diffraction method was undertaken at the Eastern Regional Laboratory.

In the course of this work several new compounds of amylose were produced and their structures established. The amylose was first put in the proper physical form by a process analogous to that used commercially for making a particularly strong cellulose fiber. This consisted in stretching films of amylose acetate in order to parallelize the threadlike molecules, then deacetylating with alkali in an alcohol solution. Examination of X-ray diffraction photographs of the regenerated amylose films showed the amylose molecules to be arranged in space in a very regular pattern or lattice. The amylose was thus "oriented" or "crystallized", and in this form was far stronger and far more resistant to water than the original amylose. Hydroxides of all the alkali metals reacted with amylose to form an isomorphous series of compounds. Amylose is a polymer of glucose, and the new alkali amyloses had the empirical formula corresponding to one alkali hydroxide molecule to three glucose residues.

Examples of another series of crystalline addition compounds of amylose were made by soaking potassium hydroxide amylose in solutions of potassium iodide, bromide, formate, and bicarbonate in 75-percent alcohol. Analysis showed one potassium halide molecule associated with two glucose residues. Identity of the space lattice dimensions for the several compounds indicated that interaction between the amylose molecules determined the structure, and that the inorganic salt occupied interstices between the amylose molecules.

Removing the alkali from alkali amyloses with ethyl or methyl alcohol left the threadlike amylose molecules in a coiled or helical form. Substituting water for the alcohol left the amylose molecules

fully extended, yet capable of at least two crystalline modifications by different sidewise packing. From the dimensions of the glucose units in the various amylose structures it appears that the glucose configuration is not fixed as it is in cellulose, but changes considerably with the environment.

ONE TYPE OF LIGNIN MADE FROM VANILLIN

The higher plants are chiefly composed of three important chemical constituents: cellulose, the hemicelluloses, and lignin. In addition, they contain lesser amounts of protein, coloring materials, vitamins, and other organic compounds together with some mineral elements. The chemical constitution of cellulose has been fairly well understood for more than 40 years, and this knowledge has been applied by large industries in the production of paper and board, of alpha cellulose used for rayon, cellulose plastics, and other cellulose products. The chemistry of the hemicelluloses has been less well understood. The valuable chemical furfural, for which important chemical uses have been developed in recent years and from which an intermediate for nylon is now made, is manufactured from the hemicelluloses of oat hulls and corncobs at the rate of about 25,000 tons per year.

The chemistry of lignin has been obscure. For more than 70 years chemists have sought to determine the chemical constitution of lignin in order to develop uses for this abundant plant substance. The problem is of prime industrial importance, because in the preparation of wood pulps for paper and chemical uses the lignin must be removed, and millions of tons of lignin in modified form must be disposed of as waste. Tremendous effort has been made to develop industrial uses for the lignin but with comparatively little success. Recently it has become the best source of the flavoring material, vanillin, but this is a comparatively small-volume product.

It has long been recognized by industry that rational use of a substance depends on an exact knowledge of its chemical constitution. In their endeavors to learn the chemical nature of lignin, chemists have broken it into fragments by a variety of chemical methods. By now all the major fragments of lignin are supposedly known, Further, it is likely that the larger fragments composing the lignin molecule are also broken down to some extent by such methods. Reasoning on these grounds, a chemist of the Northern Regional Research Laboratory, who had been successful in synthesizing tannins before he came into this Bureau, concluded that the known reactions of lignin might be explained by a rather simple structure known as the benzo-pyran ring which exists in coloring matters, tannins, and other natural plant products. This conclusion was substantiated by synthesizing a compound having such a structural formula from vanillin, perhaps the most important of the known break-down products of lignin. This synthetic compound gives all of the reactions typical of spruce or softwood lignin. But the lignin from hard woods and agricultural residues is somewhat different in that it contains chemical groupings which are not accounted for by this structural formula.

This important contribution has stimulated renewed interest and may well be a turning point in the fundamental and applied chemistry of lignin.

NEW PLANT-GROWTH REGULATORS SYNTHESIZED

A new plant-growth regulator, 2,4-dichloro-5-iodophenoxyacetic acid, has been synthesized and tested by the Biologically Active Compounds Division in cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering. This compound showed less plant-growth inhibition on dicotyledonous plants like beans than the closely related commercial weed killer 2,4-dichlorophenoxyacetic acid. Twelve derivatives of this new compound are now being tested for plant-growth regulating activity. Iodine is a constituent of the new synthetic compound, and radioactive iodine was used in synthesizing a special lot of the compound that is being used to follow the movement and study the action of a typical halogenated phenoxyacetic acid in plants.

Two other compounds of the same type (2-chloro-4-iodophenoxyacetic acid and 4-chloro-2-iodophenoxyacetic acid) have also been synthesized and tested for their ability to regulate plant growth, but the degree of growth inhibition resulting from use of these two compounds was low compared to that resulting from use of the closely related compound 2,4-dichlorophenoxyacetic acid.

Another type of plant-growth regulator has been synthesized and "labeled" with radioactive iodine. It is 2-iodo-3-nitrobenzoic acid, which was reported last year to be absorbed by both dicotyledonous plants (with inhibition of growth) and monocotyledonous plants (without effect on growth). When 2-iodo-3-nitrobenzoic acid is applied to the leaves of a dicotyledonous plant, such as the bean plant, the compound accumulates primarily in the most rapidly growing parts. Since carbohydrates are also translocated predominantly to the most rapidly growing parts of a young plant, an investigation was undertaken to determine if the growth-inhibiting action of this compound could be due to its chemical combination with carbohydrate, thereby interfering with an essential metabolic process of the young plant. The results of this investigation showed that the radioactivity resulting from presence of the "labeled" compound was associated primarily with the carbohydrate fraction of the plant. Further, the regulator compound was chemically bound to the carbohydrate fraction and could be separated from this fraction only after acid hydrolysis of the carbohydrate. This result marks the first step in learning how plant-growth regulators act in plants.

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