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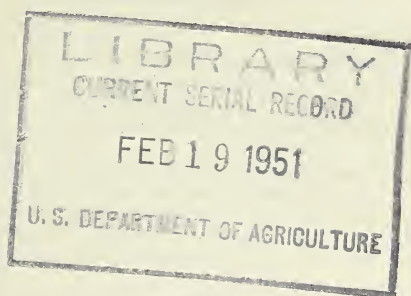
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Report of the Chief of the Bureau of Agricultural and Industrial Chemistry Agricultural Research Administration

1950



UNITED STATES DEPARTMENT OF AGRICULTURE

Report of the Chief of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, 1950

UNITED STATES DEPARTMENT OF AGRICULTURE,
Washington, D. C., September 1, 1950.

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, 1950.

Sincerely,

G. E. HILBERT,
Chief.

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INTRODUCTION

The research of the Bureau of Agricultural and Industrial Chemistry during the past fiscal year has resulted in marked progress in many projects set up to facilitate and increase the utilization of agricultural materials for foods, feeds, clothing, medicines, and fuels and other industrial products.

New equipment developed for processing cotton includes a machine for opening up baled cotton for cleaning and carding, an improved device for applying sizing to single-ply warp yarns to strengthen them for weaving, and a special loom attachment for weaving fabrics of greater density. A simple finishing treatment has been developed for making cotton fabrics easier to launder and keep clean. Three oil-seeds—sesame, buffalo-gourd, and safflower—which are not grown extensively in this country, have been investigated to determine the value of their oils for food and industrial products as an aid to the search for suitable oilseed crops for the South.

The breeding of wheat varieties having improved milling quality has been speeded up by the discovery that the milling character of wheat is related to the ratio of crude fat to crude fiber in the grain, which may be determined by analyses of small samples. New industrial products, including a lacquer, a nonsoap detergent, and a chemical that prevents the separation of lime compounds from hard water in laundering and other chemical processes, have been produced from sugars. Improved plastic materials have been made in part from constituents of animal fats. Starch sponge has been found to be useful as an absorbent in surgery. Certain micro-organisms have been found to be good producers of vitamin B₁₂, the protein factor essential for the growth of chickens. Means have been found for intensifying the flavor of maple sirup which makes it possible to produce an acceptable maple-flavored blend by adding three times as much cane-sugar sirup. Progress has been made in stabilizing the flavor of soybean oil, and whole-egg powders have been greatly improved in flavor and keeping quality.

Most of the results here reported are from research supported by funds specified in the 1950 Agricultural Appropriation Act for the regional research laboratories and for agricultural chemical and naval stores investigations.

Out of a total of 108 items grouped under 22 general headings, one relates to fundamental research on allergens, which is supported by the special research fund authorized by the Bankhead-Jones Act of June 29, 1935; one relates to natural rubber extraction and processing investigations, supported by an allotment from funds appropriated under the Strategic and Critical Materials Stock Piling Act of July 23, 1946; and one relates to the synthetic liquid fuels project, supported by an allotment from funds appropriated for studies on the production of synthetic liquid fuels from nonpetroleum sources under Public Law 290, April 5, 1944. Thirty-three of the items relate to 23 of the 46 active projects set up under the Research and Marketing Act of 1946 to encourage commercialization of new products and new proc-

esses. In each case, these particular items indicate the authority under which the work was done. Where such indication is lacking, which is true of most of the items, the work reported was done under regular Bureau projects set up under financial projects authorized by the Agricultural Appropriation Acts.

In accordance with directions from the Congress, research on certain specified fats and oils was expanded at the Northern, Eastern, and Southern Regional Research Laboratories. Work was also started under a Research and Marketing project on the production of food products from soybeans by fermentation methods. One RMA project was revised to permit plant-scale research under contract on the use of the new fungal amylase in the production of alcohol from grain. Another RMA project was expanded to permit laboratory research on the production of citric acid from molasses by mold fermentation. The Synthetic Liquid Fuels project was terminated at the end of the fiscal year because of expiration of the time during which Public Law 290 of April 5, 1944, authorized studies on the production of synthetic liquid fuels from nonpetroleum sources.

With wider utilization of agricultural materials as its objective, the Bureau must maintain, so far as it is possible, a balance between long-term research on the chemistry of agricultural commodities and short-term research on the urgent problems that continually arise in connection with the utilization of such commodities.

The major part of the Bureau's long-term research program on the country's principal farm commodities consists of fundamental scientific investigations on the carbohydrate, fat, protein, and other components of these commodities. Among the subjects of such investigations are the following:

OXIDATION OF FATS.—The oxidation of reactive constituents of fats, such as oleic acid, by air, oxygen, hydrogen peroxide, and other oxidizing agents is being studied to increase the fundamental knowledge on the mechanism of oxidation and conditions that favor intended oxidation or spontaneous oxidative rancidification of fats and oils, and also on the chemical derivatives that may be obtained by oxidation, their properties and reactions, and methods for their detection, estimation, and separation.

PHYSICAL PROPERTIES OF VEGETABLE OILS AND OF THEIR COMPONENTS AND DERIVATIVES.—Fundamental physical research is in progress on the components of vegetable oils, particularly those of cottonseed and peanut oils, to supply basic data needed by industry for the improvement and efficient operation of extraction equipment and for the processing of such oils. Studies have been made on the thermal and solubility properties of hydrogenated (hardened) cottonseed and peanut oils and of solid constituents like stearic acid, palmitic acid, and the alpha monoglyceride of stearic acid. Other studies are in progress on these and other oils. The investigations included determinations of specific heat, heat of fusion and volume change during fusion, proportions of liquid and solid at various temperatures, transformation in crystal form of solid fatty acids, and solubilities and changes of state in mixtures of liquid and solid fatty acids in organic solvents.

STRUCTURE, COMPOSITION, AND PROPERTIES OF COTTON FIBERS.—Basic research is in progress to gain additional fundamental knowledge

on the structure of cotton fibers, individual fiber properties such as length, fineness, strength, and elongation, how fiber properties are related to yarn properties, changes in tensile and elastic properties resulting from chemical or mechanical processing of cotton, nature and identity of the noncellulose components of cotton fibers, chemical degradation in the cellulose of cotton fibers resulting from various natural and artificial causes, and degree of crystallinity of cotton cellulose as it may affect the utility of cotton for various purposes.

CHEMICAL AND PHYSICAL RESEARCH ON STARCHES.—Investigations have been or are being made on the structures of starch granules and starch molecules, the fractionation of starches into amylose, amylopectin, and minor components, the properties of starch components and their derivatives, and the distinctive properties of starches from different sources. Recently the theoretically existing sugar isomaltose was separated for the first time from the amylopectin fraction of cornstarch. Experiments can now be made to learn the conditions under which this unfermentable sugar, which comprises two dextrose units linked together in an unusual way, can be broken down into a simple fermentable sugar.

ENZYMES OF AGRICULTURAL PRODUCTS.—Although most of the research on enzymes is for the purpose of obtaining new fundamental facts on their properties, chemical structure, and mode of action, much of the newly gained knowledge has proved of immediate value in practical applications, such as increasing the efficiency of enzymic methods for converting starches to fermentable sugars, reducing the splitting of vegetable oils in storage, regulating pasteurization of orange juice, reducing bin burning in underripe wheat, and synthesizing cephalins. Recent research on enzymes has explained the browning of certain fruits when peeled, the softening of brined cucumber-pickle stock, and the action of a new type of insecticide comprising certain esters of phosphoric acid. Several enzymes have been isolated in pure crystal form, and some information has been gained on the chemical structure of certain enzymes.

MICROBIOLOGICAL FERMENTATION AGENTS.—A large culture collection of yeasts, molds, and bacteria is maintained and used in basic research on the ability of particular species, strains, or mutants of various micro-organisms to produce chemical substances as the result of metabolic processes or indirectly through the action of enzymes produced by them. Studies on the biology and natural relationships of selected groups are also conducted. From the culture collection authentic cultures of particular species and strains are supplied on request to other agencies.

Other subjects of fundamental research in the Bureau include the following: Composition of soybean phosphatides; photochemical oxidation of biologically active proteins; antibiotics in plants; plant growth regulators in plant and animal products; mode of action of plant-growth regulators; isolation of pure fat acids; locating double bond in cleic acid isomers; minor proteins of milk; minor proteins of eggs; and structure, composition, and properties of wool.

Many of the short-term projects referred to are undertaken in response to requests from farm cooperatives or other agricultural groups lacking the facilities necessary for the solution of their problems. Most of the projects concern practical problems which can

often be solved by the application of known facts without need for basic research. Examples of recent short-term projects undertaken for the solution of practical problems follow:

FROTH-FLOTATION PROCESS AND EQUIPMENT FOR REMOVING NIGHTSHADE BERRIES FROM MECHANICALLY HARVESTED AND SHELLED PEAS.—This problem was of special interest to commercial growers and canners of garden peas in the Pacific Northwest, where portions of some fields were badly infested with nightshade weeds, and harvesting of the peas in these portions was sometimes not practical because of the high cost of removing the nightshade berries by hand. The process and equipment are now used commercially. More than 30 commercial froth-flotation units are now in operation.

IMPROVED KEEPING QUALITY OF COMMERCIALY PACKED AND HOME-RENDERED LARD.—To overcome lard's disadvantage, in comparison with hydrogenated-vegetable-oil shortenings, of rapidly becoming rancid because of oxidation, effective and safe fat-soluble preservatives were found, and methods were developed for preparing them from gallic and ascorbic acids. The simple expedient of adding about 5 percent of hydrogenated-vegetable-oil shortening, containing natural antioxidants (tocopherols), was recommended for retarding the development of rancidity in home-rendered lard.

PERMANENT COLOR STANDARDS FOR MAPLE SIRUP.—To make the grading of maple sirups easier and more accurate, and to remove the necessity of frequently procuring fresh standard solutions of caramel in glycerin (formerly used), permanent standards were prepared from amber-colored glass in three shades (to be supplemented with a clear solution of glycerin in water or a somewhat cloudy standard suspension of bentonite clay in such a solution). A holder was designed for comparing sirup samples with the standards, and this color comparator was made available by a commercial firm just before the beginning of the last maple-sirup season, when more than 200 were sold. The glass standards are available from the Sugar Branch of the Department's Production and Marketing Administration.

UTILIZATION OF FRUIT-CANNERY WASTES.—Investigations on the utilization of wastes from the canning of pears and other fruits are being carried on in cooperation with a farm cooperative group in Oregon and an association of fruit canners in California. Previous work in cooperation with a canning company in Washington State had indicated the probability that the liquid fraction of pear-cannery waste, separated from the fiber fraction by pressing after preliminary chemical treatment, can be used as the culture medium in an improved, continuous process for the submerged propagation of torula yeast for feed uses. Later, a laboratory process was developed for recovering sugars, in the form of sirup, from pear peelings and cores, and current work is directed toward the translation of the laboratory process into a semicontinuous pilot-plant operation. The purified sirup is useful for replacing about one-third of the refined sugar commonly used in canning pears.

Cooperative studies were carried on in a pilot plant in California on a process for making molasses and dried pomace (for use in feeds) from the mixed waste of fruit canneries. Results indicated that the process could be commercialized and that the value of the products

would make the utilization operation less costly than any acceptable method of straight waste disposal.

Additional information on the Bureau's work appears in the 363 publications issued during the year. Most of the research papers are published in non-Government scientific and technical journals. Usually a limited number of reprints are bought for distribution to seriously interested individuals and firms as long as the supply lasts. Information on newly developed processes and products is given in the specifications of the 76 Government-controlled patents granted during the year to employees of the Bureau. These patents are assigned to the Government of the United States, usually as represented by the Secretary of Agriculture. Nonexclusive licenses for their use are granted to responsible persons and firms without the payment of royalties.

A list of publications issued and patents granted is available at the Bureau's Washington office. Copies of individual publications are distributed by the issuing Regional Laboratory or the Washington Office. Copies of the specifications for individual patents may be procured from the Commissioner of Patents in Washington at 25 cents each.

The Information Division of the Bureau has supplied the public with information on the Bureau's work in nontechnical language through press releases, addresses, and contributions to radio and television programs. It has also assisted authors in the preparation of material for publication by the Department. The head of the division has addressed numerous gatherings of agricultural, trade, educational, and civic associations and exhibited new products developed through the Bureau's research. It is estimated that through the year close to 75,000 persons learned about the Bureau's accomplishments in this way.

About midway in the fiscal year, two additional assistant chiefs were added to the administrative staff of the Bureau. One is Dr. Walter M. Scott, who was director of the Southern Regional Research Laboratory for the preceding 4 years and was succeeded in that position by Dr. Charles H. Fisher, previously head of the Carbohydrate Division of the Eastern Regional Laboratory. The other new assistant chief is Dr. John R. Matchett, who was previously head of the Food Products Division of the Western Regional Laboratory and later technical adviser to the Chief of Bureau on food research.

The new appointments increased to four the number of assistant chiefs for research. Each has been charged with the coordination of a part of the Bureau's research work. C. F. Speh coordinates research on the utilization of cereal and forage crops, sugar, and special plants, including tobacco, wild plants, pine gum, and domestic sources of tannin and natural rubber. Dr. George W. Irving, Jr., coordinates research on utilization of oilseeds, poultry, and dairy and other animal products, and the fundamental research on allergens of agricultural products, biologically active chemical compounds, enzymes, and microbiological and pharmacological problems. Dr. Walter M. Scott coordinates research on the utilization of cotton, other fiber crops, and agricultural residues. Dr. John R. Matchett coordinates research on the utilization of fruits and vegetables. Henry A. Donovan is assistant chief for administration of the Bureau's business affairs.

COTTON AND COTTON PRODUCTS

New Cotton-Processing Equipment Developed

Three pieces of cotton-processing equipment under development at the Southern Regional Research Laboratory are of interest to the textile industry. One is a simple and efficient loom attachment for weaving fabrics of greater density; the second is an entirely new type of slasher, a machine used to apply sizing to cotton warp yarns to strengthen them for weaving; and the third is a machine for opening cotton in a textile mill, to aid in cleaning.

Experience of the Germans in World War II, with their Kreutzwalke attachment for weaving dense fabrics, and of investigators in this country, showed that increases above the normal number of filling yarns in a fabric is an advantage in obstructing the passage of wind and water. However, the German device placed excessive strain on the warp yarns and was deemed impractical for use in this country. To meet the need, the Laboratory's textile engineers developed a loom attachment, which was referred to in the report for 1949 in connection with the development of self-sealing, water-resistant fabrics. The design of the attachment has been simplified, and on the basis of service tests the new model is believed to be mechanically sound for industrial use. The maximum density obtainable is not limited by the mechanics of the loom or by the attachment, but only by the resilience of the yarn and the fabric construction. A normally woven fabric leaked almost 72 times as fast as a representative dense fabric when both were tested for water permeability after being previously wet with water. These close-woven goods should make cotton more competitive with synthetic fibers for outer garments, tentage, and tarpaulins, and in other fields where resistance to water and air is a factor. Aside from their water-repellent properties, the fabrics are being evaluated for dust guards in railway-car journal boxes.

The new-type slasher constitutes a progressive step in designing textile equipment on modern scientific principles. Conventional cotton-slashing equipment is fairly adequate from a production standpoint, but the quality of the sized yarns is not what is desired. Improving the quality of the sized warp will lead to greater weave-room efficiency, an important factor in textile-mill operation. The Southern Laboratory's slasher has three unique features: A new method of cooking and applying the size solution—constant circulation in an enclosed system—that avoids contamination by live steam; an original design of radiant-convection oven, using high-intensity, infrared radiation as the heat source, with over-all drying efficiencies approaching 80 percent; and an electronically controlled, direct-current, multi-motor drive system providing automatic regulation of yarn tension.

The encouraging results of preliminary tests with a machine which opens and fluffs up matted lumps of cotton into small, soft tufts, as an aid in cleaning, was mentioned in the 1948 report. During the past year, tests on the experimental model were completed, and details of the equipment are being made available to the industry.

The new cotton opener is based on the Laboratory's wartime research in which a high-capacity machine was developed for cutting low-grade, short-staple lint cotton into lengths suitable for purifica-

tion, thus insuring an adequate source of high-grade cellulose for smokeless powder. The feeding unit of this war equipment was redesigned and adapted for use in the textile industry. Essentially, the opener consists of a number of toothed gin-saw cylinders or licker-in wire cylinders arranged one above the other and rotating in the same direction. Cotton is fed by a conveyor belt into the machine, where it forms a rapidly revolving soft roll. Small tufts are pulled out of this roll by the rotating cylinder teeth, and then the tufts are removed from the teeth by means of a new doffing apparatus with metal blades.

The metal doffer, an outstanding feature of the cotton opener, appears to have potential application in cotton ginning and other fields where short fibrous materials are handled.

Requiring only 50 square feet of floor space, the commercial-size cotton opener designed by the Southern Laboratory for textile use has a rated production of 2,000 pounds per hour. It has, however, been constructed in a size processing up to 10 times this hourly rate and has been operated satisfactorily for feeding the cotton cutter.

Tests showed that use of the cotton opener does not increase neps; in fact, in some cases the card web had fewer neps when the opener was used. The spun yarns appeared loftier, and their strength and grade were unaffected. The value of the machine lies in its ability to rapidly open the cotton to a loose fluffy condition, similar to that of lint at a cotton gin before being baled, which permits improved blending and cleaning by existing textile equipment.

The principle of this opener has been described in U. S. Patent No. 2,365,793, which is available for license from the Secretary of Agriculture on a royalty-free, nonexclusive basis.

Dye Test for Thin-Walled Cotton Helps Solve Cotton-Mill Problems

The differential dyeing technique developed at the Southern Regional Research Laboratory to distinguish thin-walled cotton fibers was fully described in the reports for 1947 and 1949. This year a large number of practical applications, developed in some cases in cooperation with the Southern Laboratory, proved the expanding usefulness of this test in the cotton trade.

The test has been used in mill "trouble shooting" to solve problems associated with low-maturity (thin-walled) cotton. The results enabled the manufacturers concerned to maintain quality without appreciably increasing costs and also enabled Southern Laboratory technologists to reach conclusions of general interest to the cotton trade. For example, the test revealed that certain cottons, graded by classers as spotty, did not contain, as the manufacturer had believed, an undue number of thin-walled fibers; further, that these cottons had the same dyeing characteristics as cottons of higher grade, with which they could therefore be mixed without causing dyeing defects in the finished goods, and with a saving in manufacturing cost. In another mill, excessive numbers of prominent white specks in denims persisted even though several grades of more expensive cottons had been tried in order to eliminate them. The test showed easily that the white specks were not, as assumed by the manufacturer, mainly

the usual neps—tangled bits of highly immature fiber—but were largely tangled bits of normal fibers which faulty processing allowed to pass over into the goods. Correction of certain machine settings permitted a return to the use of normal lower-priced cotton.

The special dyeing technique is also being used in brokers' and mill laboratories. One application by brokers is for rating cottons for approximate percentage of mature fibers and for selecting bales for mill lots. The method is especially suited to aid in the selection by brokers' laboratories of even-running lots, which are of great advantage to cotton manufacturers. It is also suited to cotton-mill laboratories for the selection of cottons for particular lines of goods.

Under a Research and Marketing Act project, efforts are being made to adapt the test for more nearly quantitative correlation with cotton fineness, as determined, for example, by the Micronaire (air-flow) method, and with the usual maturity tests, particularly the caustic-soda method. Although the results obtained so far show rather good correlation, the differential-dyeing effect appears to depend upon something more fundamental than either fineness or wall thickness and does not quite measure either. In many cases, however, the dye test told more about the character of the cotton than did either of the conventional tests. For instance, differential dyeing showed clearly that large numbers of individual fibers in various experimental and commercial cottons were not uniform from end to end but were thick-walled in the middle and thin-walled at one or both ends, sometimes for as much as half or two-thirds of the fiber's length. Although such differences were previously known to exist in cotton fibers, they had not been discernible by any practical means. The way is now open for studying the effects of large proportions of such fibers on spinning quality and on specific final cotton products.

In studies of the underlying causes of the differential-dyeing effect, it was observed that the opening of bolls and drying out of cotton in the field caused submicroscopic changes which altered completely the differential-dyeing properties of the fibers. Because the test depends on submicroscopic differences in celluloses rather than specifically on wall thickness of cotton fibers, it is finding application to other cellulose fibers, particularly the rayons.

Cotton Fabrics Made Easier to Launder and Keep Clean

A simple treatment that makes cotton clothes harder to soil and easier to launder may soon lighten the washday work of housewives and aid commercial laundries. This new soil-resistant treatment is a development of the Institute of Textile Technology at Charlottesville, Va., in research conducted in collaboration with the Southern Regional Research Laboratory under a Research and Marketing Act contract with the United States Department of Agriculture.

The process consists of simply adding a small amount of a commercial compound (carboxymethyl cellulose), having the trade name CMC, to the final rinse water each time cotton goods are washed. The effect on the finish is much like that of ordinary starch, but CMC can be applied in quantities that later prevent soiling and make the cloth clean easily without making the fabric noticeably stiff. The application in effective amount—three level tablespoonfuls of CMC

per gallon of rinse water—does not change the feel of the fabrics. Use of four times this amount slightly stiffens dried cloth.

To test the treatment, CMC-rinsed and -dried samples of cotton fabric were uniformly soiled with carbon black. When washed again, they returned easily to their original whiteness. Untreated fabrics similarly soiled could not be washed cleaner than a dull, dirty gray.

Development of the treatment was an outcome of fundamental studies at the Institute of Textile Technology to determine how soil is attached to the cotton fiber. These studies showed that the soil particles are entrapped in the intrayarn and interyarn spaces and in the irregularities of the fiber surfaces. Reduction of soiling was sought by a selection of fabric-treating materials that would reduce the fiber and fabric irregularities and also act as an agent to bring the soil particles into suspension during washing. A number of compounds besides carboxymethyl cellulose, such as carboxymethyl starch, methyl cellulose, and polymethacrylic acid, function in this capacity, but carboxymethyl cellulose gives by far the most striking results.

CMC is a cellulose derivative, prepared commercially as a white powder for the wholesale market. It has been used as a thickener for textile printing pastes, as a detergent builder, and as a sizing agent. A keen interest is being shown by the laundry trade in the use of CMC for soil resistance, and the product may soon be made available in small packages for household use.

Insect-Resistant Flour Bags Tested by Industry and Army

Last year's report mentioned a method of protecting cotton flour and feed bags from insect penetration by treatment with a mixture of pyrethrins and piperonyl butoxide, developed under Research and Marketing Act funds by the Southern Regional Research Laboratory in cooperation with the Bureau of Entomology and Plant Quarantine.

Tests by several large bag manufacturers during the year confirmed the value of pyrethrins for this treatment and showed that application is practical from a textile standpoint. A problem arose, however, after about 3 months of storage of the treated flour-filled bags. The piperonyl butoxide, the oily constituent used as a synergist (activator) in the formulation, had migrated into the contents of the bag. This means that other agents that will be less likely to leave the cloth must be found. Pyrethrins by themselves are initially very effective in repelling insects when applied to cotton bagging but gradually lose their strength by oxidation or some other chemical change. Chemicals that are reported to be superior protectants for the pyrethrins and may not have the disadvantage of migrating are now being tested.

The Office of the Quartermaster General is sponsoring a test on treated cotton and paper flour bags containing a wide variety of insect-repellent formulations, including the best known activators of the pyrethrins. The Southern Laboratory applied 26 of these formulations to about 1,000 yards of cotton cloth for this work. The treated cloth was shipped to a manufacturer to be made into bags which will be filled with flour and tested for resistance to insects under Army supervision.

Chemical Test for Honeydew on Cotton Aids Cotton Industry

The cotton-textile industry was given a simple test to detect the presence of honeydew on cotton ready for manufacture. Chemists at the Southern Regional Laboratory offered this test after attention was called to the problem by members of the industry in 1949, when cotton contaminated with honeydew was encountered much more frequently than usual, especially in the case of irrigated cottons.

Honeydew is a sticky substance that is excreted by aphids (plant lice) which feed upon the cotton plant during its growth. If not detected on cotton as it reaches the mill, honeydew causes processing difficulties, particularly in the opening and cleaning steps. Yet honeydew is not easy to detect by the eye, for the sugars and other carbohydrates of which it is composed may have been dissolved or spread by rain or dew. So, quite often, honeydew remains on the cotton being processed, causing the fibers to stick to the machinery, and requiring operations to be slowed down while the clinging mass is removed. Time consumed in this stoppage means financial loss to mill owners.

In seeking a solution, chemists of the Southern Laboratory surveyed available methods for the detection of soluble carbohydrates and chose one based on the alpha-naphthol reaction as the most practical to apply for the purpose. This chemical reagent develops a dark wine-red or purple-red color when applied to water extracts of honeydewed cotton, whereas if applied to clean raw cotton it develops only a pale pink or violet color. The test requires care in its application; but it can be used by persons having no chemical training or experience and so is suitable for use as a routine check by almost anyone—mill worker, broker, or chemist—who may have need of it.

Announcement in trade journals that a mimeographed publication describing the method was available brought numerous requests for copies from the cotton industry.

Fine-Fibered Cottons Yield Stronger Yarns

Knowledge of the relations of individual fiber properties to yarn properties can have great practical importance in the improvement of cotton fabrics for specific uses. These relations, therefore, are the subject of an investigation at the Southern Laboratory. Some data which have been obtained in one phase of the work, on the influence of fiber fineness on yarn strength and elongation, as affected by twist, should lead to better cotton products for particular purposes.

Fiber fineness and fiber length are highly correlated properties, and only by means of a specially developed technique was it possible to assess the effect of fineness, distinct from that of length, on yarn properties. It was found that fiber fineness had little or no effect on yarn elongation. Fine-fibered cottons yielded yarns of higher strength than did coarse-fibered cottons in all the counts spun (16s, 24s, 36s, and 50s); and with the higher counts the differences were more marked. Fine cottons responded much more quickly to twist than did coarse cottons; that is, the yarns attained maximum strength with lower twists, and after reaching maximum strength lost strength much more rapidly with increases in twist than did yarns spun from coarse fibers.

This effect indicates that fine fibers shear more easily than coarse fibers.

Basic facts of this type are clearly important to spinners of both soft (low-twist) and hard (high-twist) single yarns. Also, they are important to cotton merchants, who, knowing in advance the effect of fiber fineness on yarn strength, can deal better with customer requests for cottons possessing the inherent characteristics that will produce the desired textiles. In addition, cotton breeders who have information about the importance of fineness can give attention to this property in their programs.

New Textile Products Developed from Modified Cotton

Two entirely different cotton products were developed recently at the Southern Laboratory from one type of chemically modified cotton, namely partially carboxymethylated cotton. The basic process for preparing both products consists in replacing cellulose hydroxyls with carboxymethyl groups by treating cottons with alkali and chloroacetic acid. One product is a highly carboxymethylated water- and alkali-soluble cotton; the other, a much more mildly carboxymethylated insoluble cotton.

While both products are salts of carboxymethyl cellulose, the different methods of preparing them result in quite distinctive properties. Each is adaptable to a particular group of uses and has attracted the attention of specific industries interested in these fields.

The soluble, partially carboxymethylated cotton is valuable for certain specialty uses. In yarn form, it can be used in intermediary processing stages, as it retains the strength of the original cotton and can then be removed by simple washing. Alkali-soluble fibers and yarns are new in this country but have been produced in England by spinning a solution of sodium alginate into a solution of bivalent or multivalent metal salt. These are used in light-weight woolen goods, novelty fabrics, and imitation astrakhan materials, and as the connecting yarns in the "string-sox" process of hosiery manufacture. In earlier work the Southern Laboratory prepared and patented similar soluble fibers by spinning solutions of sodium carboxymethyl cellulose into solutions of heavy metal salts. The carboxymethylated soluble cotton yarns have advantages over the metal-salt fibers in that their production avoids the processes of solution, extrusion, and regeneration; also, there is no heavy metal in the finished product, which keeps it nontoxic and opens up its possible use in food-packaging materials.

The second product, the insoluble, mildly carboxymethylated cotton, has greater water absorbency and a faster rate of absorption than unmodified cotton. If put into the form of the salt of a heavy metal, it has better rot resistance than unmodified cotton. This type of carboxymethylated cotton has been shown to be more resistant to soiling than ordinary cotton. There are indications that it may also be crease-resistant, and this property is being evaluated.

Partially Acetylated Cotton Shows High Resistance to Rot and Heat

Previous reports have described the distinctive properties of the versatile "new" fiber, partially acetylated cotton, under development at the Southern Regional Research Laboratory.

Among the properties before described is the high resistance of partially acetylated cotton to mildew and rotting, or degradation by micro-organisms. Acetylated twines for fishing gear, which at the time of the report for 1949 were being tested by immersion in North Carolina coastal waters, continued to perform well for 8 months, when some of the twines still retained 50 percent or more of their strength. They all lasted between 2 and 3 times as long as a tar-treated "standard" commercial twine, and at least 8 or 10 times as long as untreated cotton.

The heat resistance of acetylated cotton is proving to be of outstanding interest. This is most clearly exemplified by laundry press-pad covers of acetylated cotton, a use which subjects the product to frequent intermittent heating to a high temperature in the presence of moisture and atmospheric oxygen. The life of the treated cotton covers is increased three to four times over the life of ordinary cloth. Partially acetylated napped flannel used in press pads is also performing with superior results. Commercial production of such fabrics is under consideration by several firms.

The partial-acetylation process has been applied experimentally in the treatment of raw cotton before it is spun. This product is being evaluated for specialized uses where resiliency will be a requirement, as in garment-filling materials.

Partially acetylated cotton sewing threads, which already had proved highly resistant to rotting, were reported by a thread manufacturer to have good sewing quality. This report confirms other evidence that the treated cotton should be especially satisfactory for thread for outdoor uses.

These properties established for partially acetylated cotton apply to products made on a semicommercial scale by batch methods. A rapid continuous process under development promises to make the process more attractive to industry.

Modified Cotton Fabrics Evaluated as Ion Exchangers

The use of chemically modified cotton fabrics in the ion-exchange field, for the purification of chemical solutions, dispersions, gases, and the like, and the methods employed at the Southern Regional Research Laboratory for the production of these fabrics, were described in last year's report. In continuation of this research, several problems affecting industrial utilization are being attacked.

Attention is being directed to ways of increasing the ion-exchange capacities of the modified fabrics so that they will compare favorably with the commercial ion-exchange resins. As an example of progress along this line, it was found that by exposing aminized cotton fabric having an exchange capacity of 550 milliequivalents per kilogram to the vapor of ethylenimine an exchange capacity of about 2,500 could be obtained, as compared to a capacity of about 3,000 for the commercial exchangers used to remove acid impurities. In continued

work means will be sought to further raise this capacity and also the capacities of the other chemically modified fabrics.

A cotton fabric with acid qualities, a type of material which can be used to remove metals in ion-exchange applications, has been produced on standard textile-finishing equipment. This is the sulfoethyl cellulose fabric mentioned in last year's report.

Because the application of an ion-exchange material depends to a large extent on the acidity range in which it is effective, work was done to determine this range for the various ion-exchange cottons. This property is related to the acidic or basic strength of the exchanger and was measured by the pH at which the exchanger exhibited half its maximum capacity. When evaluated in this way, sulfoethyl cellulose was found to be a cation-exchanger of high acid strength while carboxymethyl cellulose and the succinic-acid-half ester of cellulose were found to have the properties of weak acids. Phosphorylated cotton behaved like a strong dibasic acid. Aminoethyl cellulose, a primary amine, and diethylamino cellulose, a tertiary amine, were found to be weakly basic anion-exchangers. On treatment with methyl iodide, diethylamino cellulose was converted to a quaternary derivative of high basic strength.

Additional samples of these ion-exchange fabrics have been sent out for evaluation by interested agencies. In the continuing pursuit of all the possibilities opened up in this field, still other cellulose derivatives that appear promising will be investigated, and the modified cottons will be produced and evaluated.

Microscopical Techniques Used for Measuring Swelling and Closing Capacity of Cotton Fibers

In two lines of fundamental investigations using microscopical techniques at the Southern Regional Research Laboratory, knowledge has been gained of the comparative swelling in water and closing capacities of various types and varieties of cotton. The combined results of the studies, by confirming that preference should be given to thin-walled fibers, have aided a long-range research project on the development of special self-sealing cotton fabrics.

One of the microscopical techniques worked out under an RMA project gives research workers for the first time a reliable estimate of cross-sectional swelling of fibers from different varieties of cotton and at different stages of cell-wall development. Applications of the technique showed that cross-sectional swelling of fibers in water varied from 21 to 34 percent, with a mean swelling of about 30 percent, as compared with 65 percent for raw flax and a sample of viscose rayon and no lateral swelling for nylon.

Microscopical comparisons of the swelling of mature (thick-walled) and immature (thin-walled) fractions of cotton samples showed that, although swelling of the two types of fibers is statistically different, the differences are too small to explain the advantage which fabrics manufactured from fine or immature cottons have over those produced from mature cotton to resist the penetration of water. Microscopical comparisons of the swelling of samples from six varieties of cotton did not offer conclusive evidence for the selection of any one variety over another for the manufacture of self-sealing fabrics. Swelling

of cotton in water is a commonly recognized phenomenon, but little information has been available on the extent of this swelling.

In a microscopical evaluation of the comparative closing capacities of different cottons it was learned that there are nearly twice as many fibers in a given weight of immature as in a like weight of mature fibers and that the spaces between immature fibers are consequently smaller. This is probably the major factor contributing to greater water resistance of fabrics made of fine-fibered cottons.

More Learned About Physical Properties of Cotton Fiber

In fundamental cotton-fiber research at the Southern Laboratory, the length-strength relation was investigated as one step in an extensive study of the relations between individual fiber properties. The results showed that the strength and elongation capacity of fibers within a sample extended over a wide range. In general, strength and elongation increased with fiber length within the sample and within a group of samples of different lengths. A proposed strength index, determined from the three length-groups of maximum weight, was found to be representative of the average strength of all lengths. A selection of fibers from these groups for testing reduces the number of specimens required for a reliable test and simplifies sampling procedures when a comparison of chemically treated cottons with raw cottons must be made. There is some evidence that the fiber strength determined from such a selection will be better correlated with yarn strength than fiber strength determined with conventional sampling.

A report from another research agency that mechanical processing produced chemical degradation in cotton led to an investigation at the Southern Laboratory of the individual fiber strength, elongation at breaking point, uniformity, and viscosity of processed cottons. No evidence of chemical degradation was found in processed cotton judged by viscosity data determined by the laboratory's adaptation of the cuprammonium fluidity method. Nor did mechanical tests of fiber properties show that the processed cottons suffered any significant damage. Changes in properties during processing, but not considered as degradation, were decreased elasticity and increased uniformity between fibers of the same sample. Basic studies of this type will be continued to obtain definite evidence on the effect of mechanical processing over the entire range of fiber properties.

OILSEEDS AND THEIR PRODUCTS

Histories of Stored Cottonseed Show How To Reduce Loss of Oil

To avoid as much as possible spontaneous heating of cottonseed in storage, which is generally accompanied by rapid deterioration, it is customary in nearly all mills to segregate cottonseed lots on the basis of their moisture contents. Nevertheless, serious difficulties sometimes arise, especially in humid climates. This is true even when the seed has a relatively low moisture content. Whenever receipts accumulate so rapidly that high-moisture seed must be stored, more serious difficulties are encountered.

Realizing that accurate knowledge of the changes that take place in stored cottonseed under different conditions might make it possible to substantially lessen the difficulties, the Southern Laboratory has made a thorough study of the behavior of cottonseed of various moisture contents stored under conditions that normally prevail at oil mills. This study has been carried out in a series of experiments in cooperation with oil mills from 1944 through 1950 and has paralleled the Laboratory's investigations of chemical treatment as a means of reducing the deterioration of cottonseed during storage.

The study revealed that neither the production of heat nor the rate at which free fatty acids are formed in stored cottonseed is entirely dependent on the initial moisture content. They are also dependent on the initial content of free fatty acids in the seed. Furthermore, a reduction of the moisture content by sustained aeration does not stop the rapid rise in free fatty acids. During this study the Laboratory also observed that the oil content of cottonseed apparently decreases during storage. There were increases in refining loss, which appeared to be proportional to the quantity of free fatty acids developed in the seed.

As the extent of losses in storage seems to be directly dependent on the initial content of free fatty acids, as well as on the initial content of moisture, both of these factors—not just the moisture—should be considered when seed is segregated for storage in a mill. Consideration of the moisture content alone does not permit the most effective segregation.

Cottonseed Solvent-Extraction Industry Aided

During recent years, several commercial plants in the United States have been processing cottonseed by continuous solvent extraction. Gradually this method—already highly successful with soybeans—is achieving a definite place in the cottonseed processing industry. It offers several advantages over mechanical processing, the main one being a higher recovery of oil.

For a number of years the Southern Regional Research Laboratory has been attempting to solve important technological problems involved in adapting the solvent-extraction process to cottonseed. Some have been eliminated in pilot-plant experiments. Data which enable processors to select the temperature and pressure needed to efficiently remove the solvent from mixtures of oil and solvent in various proportions have been furnished to oil mills, as described in the 1949 report. The proper control of heat during removal of the solvent has been found to reduce the fixation of color in the oil. When heat is properly controlled the finished refined oils are equal in quality to high-grade hydraulic-pressed oils.

Another problem which has plagued cottonseed processors using solvent extraction has been the effect of moisture on cottonseed flakes. Recent studies at the Southern Laboratory have shown that cottonseed having a low moisture content yields flakes containing an excessive amount of very fine material, whereas high-moisture cottonseed yields soft flakes. Both types tend to pack within an extractor and are difficult to extract because they do not permit the solvent to flow freely.

Further investigation has shown that with flakes having around 9 percent moisture there is very little of the objectionable fine material. Such flakes also are tough enough to withstand packing in an extractor and to permit free percolation of solvent. Thus, they make efficient extraction possible. To obtain flakes of this moisture content, the seed should have 10 to 12 percent moisture. Wetter seed should be dried to this moisture content after removal of the linters. Drier seed should be moistened to obtain the optimum 10 to 12 percent level.

A moisture-equilibration technique has been worked out for increasing the moisture content of dry cottonseed to the optimum level for the production of flakes most suitable for solvent extraction. This technique was applied successfully to cottonseed used in pilot-plant solvent-extraction investigations at the Southern Laboratory. It has direct application to use in improving over-all operations for a solvent-extraction process, particularly in an area where the seed is extremely dry.

These findings were made available to industry at a series of working conferences with the superintendents and managers of cooperative cottonseed-oil mills. Four such conferences were held during the year. They gave engineers of the Southern Laboratory an opportunity to discuss the entire category of solvent-extraction problems directly with mill operators. Technical assistance was given, and specific recommendations for the solution of their problems were made at the request of the operators.

Whole Cottonseed Solvent Extracted

The mechanical removal of linters from cottonseed prior to extraction of the oil, although universally practiced in the industry, is one of the most expensive operations in cottonseed processing. The hulling of delinted cottonseed and the separation of the meats from the hulls present some mills with difficult operating problems and result in loss of valuable oil in the hulls.

With these apparent shortcomings of the usual processing methods in mind, a preliminary study was made of the technical and economic feasibility of a process that would have as its object the solvent extraction of the oil from cottonseed prior to the removal of linters, and treatment of the residue to effect separation of meal, hulls, and linters. This project has been carried out by the Cottonseed Products Research Laboratory of the Texas Engineering Experiment Station at College Station, Tex., for the Cotton Research Committee of Texas under an RMA contract supervised by the Southern Regional Research Laboratory.

A process was developed by laboratory and pilot-plant experimentation involving removal of first-cut linters, after which the remaining whole seeds are rolled and extracted with solvent. Existing commercial methods are used to separate the meal, hulls, and remaining linters from the extracted residue. Although operating costs are slightly higher (about 55 cents per ton of seed) than for ordinary solvent-extraction processes, the value of the products obtained by this process is also a little more, indicating that the new process might be able to compete with the solvent-extraction processes now in use.

However, there is no additional profit to compensate for the greater initial investment required and for the added expense of marketing new products such as the hulls-lint fraction.

Higher quality oil and meal were also produced without removing any linters from the seed. Such a procedure is expensive, but this process might merit further consideration if new and more valuable uses for the hulls-lint fraction could be developed.

Cottonseed-Fractionation Process Improved—Increased Yields Obtained

Improvements were made in the unique cottonseed-fractionation process previously developed at the Southern Laboratory under an RMA project. This process produces a meal essentially free of oil, pigment glands, and hulls, and having very high protein solubility. Limited feeding tests with chickens and rats indicate that the meal is highly nutritious. Pigment glands are a byproduct for which possible uses are being sought. A high-grade oil is also produced.

In this fractionation process, as used at present, flaked cottonseed kernels are disintegrated into a meal-solvent slurry, using commercial hexane, to detach meal tissue from the pigment glands. The disintegrated solids are separated from the oil-solvent mixture by a new differential settling method. Most of the meal is recovered from the separated solids by passing them through a high-speed, continuous horizontal centrifuge. That leaves the pigment glands concentrated in a relatively small portion of the original meal from which they, in turn, are recovered. The oil is obtained by evaporating the solvent from the oil-solvent mixture.

Considerable quantities of cottonseed products had been produced in batch lots by this method in a pilot plant representing semicommercial equipment. On the basis of this pilot-plant work, a complete engineering evaluation of the process was made and flow diagrams were published.

Although the process at this stage was promising in many respects, the 50- to 55-percent yields of pigment-free meal obtained were not attractive for commercial adaptation of the fractionation technique. Recent efforts, therefore, have been directed toward substantially increasing the yields. In experiments on a somewhat smaller scale and with a closed-circuit disintegrating-screening system, in which meal particles are repeatedly and alternately disintegrated and screened, yields of 62 percent pigment-free meal have been obtained. Calculations indicate that in larger scale continuous operations of this kind the yield of such meal might exceed 75 percent, a figure which should attract commercial interest.

In recent studies emphasis also has been placed on the possible use of a combination solvent-extraction and fractionation system. Solvent extraction removes the oil and yields a solvent-moistened meal from which a purified meal, free of pigment glands, can readily be produced by fractionation. Preliminary cost analysis indicates that a combination solvent-extraction-fractionation plant may be commercially feasible and desirable, but additional study is needed.

Controlled Processing Gives Better Cottonseed Meal

Authorities on nutrition have recommended that not more than 9 or 10 percent of cottonseed meal be used in the diet of swine and not more than 5 percent in the diet of chickens. As about 75 percent of the entire output of protein concentrates is used in feeds for these animals, it is evident that the potential market for cottonseed meals is greatly curtailed.

The nutritional value of cottonseed meal depends chiefly on the quality of the protein (whether soluble or insoluble) and on the content of free gossypol. Cottonseed meals with a high content of soluble protein nitrogen may have a high nutritional value, while meals with a low content of free gossypol (that is, gossypol that can be readily extracted by aqueous acetone and other solvents) are more nutritious than meals containing larger amounts of free gossypol.

To obtain information to guide the planning of research to improve the nutritional quality of cottonseed meal, the Southern Laboratory, under an RMA project, has determined the soluble protein-nitrogen and free-gossypol contents of cottonseed meals now on the market. Seventeen different meals were obtained from cooperating screw-press, hydraulic-press, and solvent-extraction plants. The free-gossypol content of these meals varied from 0.01 to 0.58 percent; the soluble protein-nitrogen content, from 8 to 71.6 percent.

It is evident from the results that some of the meals examined may have had high nutritive value. The survey clearly demonstrated that processing procedures greatly influence the chemical properties of cottonseed meals. It also emphasized the advisability of investigating the chemical and nutritional properties of cottonseed meals prepared under controlled processing conditions.

Accordingly, the Southern Laboratory has intensified its efforts to assist the cottonseed-processing industry in manufacturing meals of the highest possible nutritive value by determining the most satisfactory conditions of processing. Screw press, hydraulic press, and solvent-extraction procedures are being studied. Investigations so far have shown that excessive heat and pressure greatly reduce the amount of soluble nitrogen—to as low as 7 percent in the meals, compared with as much as 80 percent in the unprocessed cottonseed. Certain conditions of processing also affect the free-gossypol content of the meals produced.

During this study, screw-pressed meals have been prepared under carefully controlled processing conditions. The maximum temperature and length of time in the cooker and energy input to the press were varied over wide ranges. Interesting results were obtained with cooking temperatures much lower than those normally used in screw-press operations.

Extensive nutritional studies are being made with these experimental products. When meals with an optimum nutritional value have been found, pressroom conditions can be easily adjusted to produce meals having the desired chemical and nutritional properties.

Cottonseed-Meal Glues Developed for Plywood Manufacture

The potential utilization of cottonseed meal or cake in the preparation of plywood glues has been reported for a number of years in the patent literature. These patents, however, deal primarily with glue formulations; they do not give instructions for the preparation of plywood for the use of cottonseed-meal glue or data on the shear strength of the glue joints. This information is necessary in order that industry may determine the feasibility of using these meals in the preparation of plywood glues.

During the year the Southern Regional Research Laboratory developed practical data on the formulation of cottonseed-meal glue and its properties, on processing conditions as they affect the shear strengths of cottonseed-meal-glue joints, on the effect of urea-formaldehyde solvent for cottonseed meal, and on the effect of processing conditions on the shear strengths of glue joints. Cottonseed-meal glues were compared with other water-resistant glues as to the shear strengths of glue bonds and the viscosity, or "working" properties, of the glues. The data will make it possible to evaluate these glues for industrial uses. The tests show interesting possibilities for cottonseed meal in the adhesive field, provided availability of meal and other economic factors justify such use.

Cold pressing of the plywood panels followed by hot pressing of the panels for an optimum length of time produced a joint of cottonseed-meal glue having satisfactory shear-strength and wood-failure values. Hot pressing at 237° F. for a 10-minute period gave the strongest glue joint. It had a dry shear strength of about 400 pounds per square inch, and in 70 percent of the samples tested the wood broke before the glue joint gave way.

Dispersion of the protein contained in the meal in a water solution of urea, followed by addition of formaldehyde, produced a glue which yielded satisfactory shear-strength and wood-failure values when used to prepare glue joints. The glue, which has a slightly basic reaction, is of particular interest for applications that require minimum discoloration of the veneer.

Glues prepared from unheated, solvent-extracted cottonseed meal compared favorably with commercial casein glue and peanut-meal glue, each glue mix and glue line being prepared as recommended to give maximum shear strength. Glues prepared from unheated, solvent-extracted meals gave stronger bonds than glues prepared from cottonseed meals made by other processes.

The viscosity of cottonseed-meal glues can be adjusted to desired values by changing the concentration of alkali and by adding trichloroacetic acid to the glue. This makes it possible to increase the "working" life of the glue, so that the time of the gluing cycle in a plant can be adjusted most advantageously.

New Combination Products of Gossypol Supplied to Research Laboratories for Pharmacological Investigations

Efforts to increase the availability of gossypol for evaluation for possible uses were continued with considerable success.

Gossypol is contained in pigment glands that are embedded in the seeds of cotton. Heretofore, most of the gossypol used in experiments at the Southern Laboratory, or furnished for investigation elsewhere, was prepared by extraction from pigment glands and subsequent purification. The laboratory has now found that relatively pure gossypol can also be prepared in high yield from the methylethyl ketone extract of oil-free cottonseed flakes.

As gossypol is somewhat toxic to chickens, swine, and other non-ruminants, its presence limits the quantities of cottonseed meal that can be fed to such animals. A cottonseed meal having superior nutritive value is obtained when gossypol is eliminated from the seed either by solvent extraction or by complete removal of the pigment glands containing the gossypol. If all the cottonseed meal now being sold were processed for feed with separation of gossypol, it is estimated that as much as 50 million pounds of gossypol would be available each year for other uses. Recovery and utilization of this byproduct could materially increase the value of cottonseed to both the farmer and the processor.

Gossypol is believed to be a naphthalene derivative. It contains aldehyde and phenol groups, and, because the compound is somewhat toxic itself, its derivatives might be valuable for such products as antiseptics and insecticides.

Biological research on this material and its application to new uses have been difficult, however, because gossypol is insoluble in water. For this reason, the Southern Laboratory in 1949 developed a water-dispersible form by combining gossypol in alkaline solution with peanut protein.

Because of the interest shown by research agencies in this compound, other water-soluble derivatives have been prepared by taking advantage of the reactivity of gossypol in alkaline solution. Slightly alkaline water solutions of gossypol form combination products with such substances as proteins, amino acids, sugars, and starch. After the alkaline solutions have been neutralized with acid, the water is removed by freezing and vacuum evaporation. The resulting products are soluble in water.

Seven research laboratories have been supplied with such water-soluble gossypol combination products. Pharmacologists are studying the properties of these compounds to aid in determining the types of gossypol derivatives that might have industrial or medicinal value. Further information is needed concerning the types of gossypol combination products that occur in cottonseed—particularly those that are toxic to farm animals. A study of the properties and reactions of gossypol derivatives is expected to aid in supplying such information.

An idea of the physiological action of water soluble combination products of gossypol, as compared with isolated gossypol, was obtained by testing the materials on goldfish at the Southern Laboratory. The isolated gossypol was not toxic at all, because it did not dissolve in the water, but all the other compounds were toxic in varying degrees. Some were toxic in concentrations as low as 1 part to 100,000 parts of water. In such a test they were even more toxic than pigment glands, which contain gossypol in its natural and soluble state.

Improved Emulsifying Agents Made From Cottonseed and Peanut Oils

The manufacture and use of monoglycerides began less than 15 years ago. Monoglycerides, generally made by heating a mixture of fat and glycerin in the presence of a small amount of catalyst (usually soap or water); are good emulsifying agents for use in both food and nonfood products. Special shortenings containing small amounts of technical-grade monoglycerides are widely used in cakes and other baked goods because they make it possible to incorporate a higher percentage of sugar in the dough than is possible with ordinary shortening. Monoglycerides are also important ingredients in margarine and many other foods. Technical-grade monoglycerides have become increasingly important in the preparation of such nonfood products as cosmetics, industrial oil-water emulsions, and special detergents. Indeed, developments in the use of monoglycerides have been more rapid than the acquisition of scientific knowledge on them.

Investigations at the Southern Laboratory under an RMA project on the production, purification, and utilization of monoglycerides from cottonseed and peanut oils have made available valuable information which has placed the technology of monoglycerides on a more scientific basis and provided data necessary for their increased industrial utilization.

Usually technical-grade monoglycerides made from these oils contain only 30 to 60 percent of the desired ingredient. Work at the Southern Laboratory showed, for the first time, just what factors govern composition and to what extent they can be controlled. The content of monoglycerides in a reaction product was found to follow statistical laws and to depend solely upon the amount of glycerin dissolved in the fat, when the reaction was allowed to proceed to completion. At a temperature of 395° F. about 3 hours was needed to complete a reaction, but at 485° F. only 15 minutes was needed. Excessive time of heating during preparation is undesirable.

Diglycerides are formed during the preparation of monoglycerides, and it was commonly assumed that the former contributed to the emulsifying properties of the technical-grade products. It was found, however, that diglycerides are less than one one-hundredth as active as monoglycerides.

Because triglyceride vegetable oils like those of cottonseed and peanut contain compounds of glycerin and certain fatty acids, it is possible to prepare various types of monoglycerides from them. The different types of monoglycerides producible from cottonseed and peanut oils were made, and their emulsifying powers were determined by measuring their ability to lower interfacial tension between oils and water. On an equal-weight basis, the surface activity of monoglycerides like monomyristin, monopalmitin, and monostearin increased in proportion to the decrease in the weight of their molecules when these compounds were used with edible oils. Under similar conditions mono-olein and monolinolein behaved substantially like monostearin. The addition of about 1 percent of monostearin to a vegetable oil reduced the interfacial tension between the oil and water to about half its original value.

When the tests were made with mineral oils, monolinolein, which is the predominant compound in technical-grade monoglycerides prepared from cottonseed oil, proved to be the most surface-active of the compounds tested. When 0.15 percent of monolinolein is added to mineral oil, the interfacial tension between mineral oil and water is reduced to less than one-tenth of its original value. These results indicate that a monoglyceride prepared from cottonseed oil is the best compound of its type for forming mineral oil-water emulsions. When monoglycerides produced from oils like coconut oil were tested with mineral oil, they were inferior to monoglycerides produced from cottonseed and peanut oils.

If the accompanying glycerin, diglycerides, and triglycerides, which are always present in technical-grade monoglycerides prepared by interaction of a fat and glycerin, are removed, the concentration of monoglyceride is doubled or tripled, and the product becomes more acceptable for many uses. Successful procedures were developed for the purification. It was found that the concentration of uncombined glycerol in technical-grade mixtures could be reduced to about 0.4 percent by dissolving the original mixture in a hydrocarbon (commercial hexane), adding water equivalent to twice the amount of free glycerin, and separating the hexane solution.

Production Problems Solved for Peanut-Butter Industry

Although the practice of preventing oil separation in peanut butter by the addition of stabilizing substances, such as hydrogenated peanut oil, has been widespread for some time, the industry has had little or no information on the mechanism by which these substances prevent oil separation, and none could be found in the technical literature. Many peanut-butter processors found that incorporation of seemingly correct amounts of hydrogenated peanut oil, when using large-capacity grinders occasioning high frictional heat and often smaller grinders producing somewhat less frictional heat, did not always prevent oil separation.

This was one of the first problems attacked by the Southern Laboratory in its study of peanut-butter processing which was started in 1949 under an RMA project. This study has provided some very worth-while information.

In efforts to learn the mechanism by which hydrogenated peanut oil prevents oil separation, the specific heats and volumes of both "stabilized" and "unstabilized" peanut butters were determined by cooling both slowly and very rapidly over the range -40° to 80° C. The results indicate that the stabilizing effect of the hydrogenated peanut oil depends to an appreciable extent on the crystalline condition of the hard (hydrogenated) fat prior to its incorporation in the butter, and that the optimum stabilizing effect can be achieved only if the greater part of the hard fat remains in, or reassumes, its original crystalline condition on completion of the processing operations and if the hard fat is uniformly dispersed throughout the peanut butter.

These findings make it possible to select a hydrogenated peanut oil for commercially "stabilizing" peanut butter on the basis of its physical properties, and indicate the exactness of temperature control that should be exercised during incorporation or other stages in the proc-

essing, so that the preexisting crystalline form of the stabilizer will be contained in the final product.

The thermal (calorimetric) data obtained in this investigation are of value to the peanut-butter industry in connection with the problems of dissipating excessive heat in peanut butter resulting from processing and shrinkage in volume, due to cooling after packaging, which produces the appearance of short measure.

When peanut-butter samples prepared under a wide range of processing conditions in the pilot plant were stored at 80° F., periodic examinations showed the products were relatively stable, had good resistance to oxidative rancidity, and satisfactorily retained their original taste and odor for 6 months or longer. The data obtained so far do not indicate that the addition of salt for flavoring and hydrogenated peanut oil for preventing oil separation, or any roasting condition, accelerates the deterioration of peanut butter.

General processing information has been obtained which is of interest to peanut-butter manufacturers and useful for controlling the quality of peanut butter and gaging the efficiency of specific operations. For example, facts are now available on the effects of roasting and other processing steps on oil content and free-fatty-acid content of the kernels, on the oil content of separated peanut skins, on the percentages of peanut halves, germs, and skins resulting from the blanching operation, and on the percentage of unblanched and undesirable material remaining with the peanut halves after the blanching operation.

Advances Made in Peanut-Meal and Protein Production

The Southern Laboratory made considerable progress in the pilot-plant production of high quality peanut meal and protein.

A light colored peanut meal suitable for food and feed products, as well as for protein production, can be obtained by removing the skins from the kernels or by extracting the color from the skins prior to grinding the kernels for solvent extraction. Skins may be removed to a large extent by a mechanical process after the kernels are dipped in water and dried. Color in the skins may be removed by treating the kernels with a dilute lye solution. These two processes, described in previous reports, are the only effective ones known for preventing an objectionable color in peanut meals. Both processes are applicable to U. S. No. 1 peanuts and are now being tested for use with other grades. Particular attention was given to the lye process. Optimum processing conditions have been determined, and improved techniques developed, for carrying out this treatment on a pilot-plant scale. Useful data have been assembled for making the operation continuous. Similar information was obtained for the water-dipping process, in which a number of factors interfere with the removal of the last few percentages of skins. The most important interfering factors have been determined in small-scale investigations, and progress has been made toward their elimination. As soon as optimum conditions are established for the water-dipping process and its operation has been made continuous, both processes will be evaluated and compared from the standpoint of cost and product quality.

The water-dipping process has the obvious advantage that it actually removes the skins and thus is more suitable for producing

peanut meal or protein destined for food uses; also, the oil and protein losses are lower. The lye process, on the other hand, is more effective in removing skin color from meal or protein intended for industrial uses, especially in the case of lower-grade peanuts. The skin content of solvent-extracted peanut meal made from lye-dipped kernels is approximately 7 percent. This so changes the physical character of the meal that screening, centrifuging, pressing, and drying the residue from protein extraction are decidedly facilitated.

More than 500 pounds of light colored, high quality peanut protein was prepared from solvent-extracted meal obtained from lye-dipped kernels. This protein was suitable for use in the Laboratory's fiber investigations, as well as for evaluation by other agencies interested in both nutritional and industrial applications. Production of this quantity permitted a thorough study of the process for peanut-protein recovery, and led to several important improvements. Larger yields were obtained by an improved method of countercurrent peptization of the meal, using either screens or a continuous horizontal centrifuge to remove the coarse undissolved (spent) meal from the peptized liquor. Improved procedures also were developed for dewatering and final drying of the spent meal, which then is suitable for animal feed. Commercial size centrifugal machines have been used successfully for final clarification of the peptized protein liquor and for removal of the protein.

New Data Help Industry Evaluate Process for Peanut-Fiber Production

The preparation of fibers from peanut protein has been reported for a number of years in both the United States and England. The Southern Laboratory has successfully made such fiber in experimental lots on equipment similar to that used in the commercial production of rayon. At the close of last year, however, pilot-scale data on the manufacture of the peanut protein fibers were not generally available to persons in this country interested in the feasibility of producing peanut fiber on an industrial scale when a sufficient supply of suitable peanut protein becomes available commercially. Nor had basic information on the chemical reactions involved in making the fiber been reported. Steps were therefore taken to supply this information.

The Southern Laboratory constructed a pilot-scale fiber-spinning machine, supplied an industrial firm with peanut-protein yarn for knitting into a textile fabric, and determined the mechanism of the interactions between isoelectric peanut protein and alkali chlorides in aqueous solutions, which are representative of conditions during the preparation of fibers from peanut protein.

The capacity of the pilot-scale fiber-spinning machine was such that the quantity of fiber extruded per unit time per spinnerette was equal to plant-scale output. Fiber strands or yarns consisting of 480 continuous filaments have been produced at a capacity of 300 grams of fiber per hour per spinnerette. The data obtained for a machine having this capacity should be useful to commercial firms interested in the potential production of protein fibers. Besides, an increased quantity of fiber has been made available for evaluation alone or in blends with other textile fibers.

The peanut-protein fibers produced by this process had about three-fourths of the dry strength of wool, a cream to white color, and a soft feel. The yarns were successfully knitted by an industrial firm to make a textile fabric.

The interactions of peanut protein and the alkali chlorides in aqueous solutions, as determined by electromotive-force measurements, were found to be dependent on the salt and protein concentrations and the temperature of the solutions. The effect of peanut protein on the electromotive force of aqueous alkali chloride solutions seems to be greater than can be accounted for by chemical interaction. It appears that certain attractive forces, which in the case of these interactions seem to be associated with adsorption, exist between the salt and the protein.

Three Potential Southern Oilseed Crops Evaluated

Restriction of cotton acreage in the past and the possibility of such restriction in the future has stimulated the search for suitable replacement crops. Reduction in cotton acreage not only reduces lint production, but also the production of cottonseed, which is processed into needed oil and meal. Therefore efforts to find a suitable replacement crop for cotton have centered largely around oilseeds. Suitable oilseeds could provide a profitable crop for the farmer and a source of raw material to keep cottonseed-oil mills operating at times when they would otherwise have to curtail production or shut down entirely. Such oilseeds could also supply the oil and meal lost as a result of reduction in cotton acreage.

Several southern and southwestern State agricultural experiment stations and private research organizations have investigated various oilseeds for the past several years in their search for a replacement crop adapted to the Cotton Belt. Of about a dozen oilseeds investigated, sesame seems most likely to meet the requirements of both the farmer and the processor.

Sesame

The dearth of published technical data and information and the interest of plant breeders, processors, and end-product users in sesame and its derived products led the Southern Laboratory to undertake an intensive investigation of the seed of some of the newly developed varieties of this plant. This investigation, the results of which are being published in a series of 12 articles in technical journals, included a thorough analysis of the seed, oil, meal, protein, and minor components of the oil. Several of the oil components, which are not found in any other oil, have special industrial value.

The seed contains about 50 percent of oil and 20 to 25 percent of protein. It is free-flowing and stores well for long periods, even in tropical climates. It requires no hulling or preparation other than cleaning prior to being milled into oil and meal. It is readily processed by ordinary oil-milling equipment, including hydraulic and continuous-screw presses, and by forepressing and solvent extraction.

The crude oil is very light in color, in this respect resembling refined cottonseed or peanut oil. It is low in free fatty acids, generally less than 2 percent, even when produced from seed stored for a year

or more. It is readily refined and by the usual methods can be bleached to a nearly water-white oil and hydrogenated to yield a hardened fat of shortening consistency. The oil varies in stability at different stages of processing, but the stability is generally as high as, or higher than, that of other "soft" or yellow oils. After being hydrogenated to shortening consistency, it exhibits extremely high stability. Variation in the stability of sesame oil at different stages of processing has been found to be correlated with its content of sesamol, a phenolic hydrolysis product of sesamol, one of the minor constituents of sesame oil. Sesamol is removed from the oil by alkali refining and deodorization and is formed from sesamol upon bleaching the oil with certain clays or carbons and upon hydrogenation. Stability of the oil increases with the sesamol content. Crude sesame oil contains a compound called sesamin, which is itself relatively inert chemically but acts as a powerful synergist in increasing the effectiveness of pyrethrin as an insecticide.

From the standpoint of easy processing and utility as a food, sesame oil has many advantages in comparison with other common vegetable oils.

Meal produced in the processing of sesame seed contains 50 to 55 percent protein. It is a good stock feed. As the production of sesame seed in the South increases it may be expected also that relatively large quantities of oil-free sesame meal will be available for possible utilization in food or industrial products.

Chemical analysis of sesame protein showed that it is similar to cottonseed protein with respect to such properties as solubility in aqueous media, viscosity of alkaline dispersions, and color. The protein can be isolated from oil-free sesame meal by extraction with a solution of sodium hydroxide at pH 10.5 and precipitation at pH 4.0 by addition of sulfuric acid. The yield represents about 50 percent of the total nitrogen content of the meal. The viscosity characteristics of alkaline sesame-protein dispersions indicate that the protein probably can be used in making certain types of glues and sizes for uses in which color is not important.

Buffalo gourd

The buffalo gourd, *Cucurbita foetidissima*, is a wild plant native to the Great Plains, southwestern United States, and northern Mexico. It is known to be growing wild in the States of South Dakota, Nebraska, Kansas, Missouri, Oklahoma, Texas, New Mexico, Colorado, Utah, Arizona, Nevada, and California and in Mexico as far south as Guanajuato. This gourd is a long-lived perennial vine which grows to be 8 to 10 feet long and, in a good location, can occupy 300 square feet. Twenty or more stems usually emerge from a single root, which may attain a size of 6 feet in length and 12 inches in diameter.

Because of interest in its seed as a possible crop for the southwestern drylands, the buffalo gourd has been evaluated as to its utility as a source of oil and meal.

The cleaned but unhulled seed contained 9.6 percent moisture, 31.7 percent protein, 26.5 percent crude fiber, 4.8 percent ash, and 24.3 percent extractable lipids (crude fat). After being extracted with a hydrocarbon solvent, the meal contained 8.6 percent moisture, 42.0 percent protein, 35.2 percent crude fiber, 7.98 percent ash, and

0.36 percent residual lipids. Although both the seed and extracted meal are high in protein, they are also high in ash and crude fiber and very low in total sugar. Consequently, the seed would have to be hulled prior to extraction, or the meal processed further to make it suitable as a stock feed.

The oil had an iodine value of 136.1, which places it in the semi-drying class along with, but slightly above, soybean oil. The components of the oil in percent, calculating the fatty acids to glycerides, were: Olein 23.0, linolein 65.3, diene glycerides 0.37, triene glycerides 0.66, tetraene glycerides 0.02, myristin 0.17, palmitin 6.13, stearin 2.22, C₂₀ and higher glycerides 0.55, unsaponifiable matter 1.53.

The crude oil was very dark and exceedingly resistant to bleaching. Refining, bleaching, and deodorizing gave a bland oil with good stability and no tendency to revert in flavor. However, the color at each stage of processing was inferior to that of other common edible oils, such as cottonseed, peanut, and soybean. Hydrogenation to shortening consistency gave a product of excellent stability (250 hours by the active-oxygen method), but this, too, was inferior in color to other common hydrogenated oils. The abnormally dark color of the crude and processed oils may be attributed to alteration of the original pigments of the seed as a result of weathering of the gourds in the field before harvest. No doubt the color quality of the oil can be improved by harvesting the gourds and drying them under shelter instead of in the field.

Safflower seed

Safflower seed has been grown in small volume in the West and Southwest for a number of years, but the acreage devoted to it expanded very little until the development of new and improved varieties at the University of Nebraska. Several of the new varieties are being grown on a fairly large scale in California, western Nebraska, and eastern Colorado, and on a smaller scale in the south-western dry lands. During World War II the seed was grown and processed in New Mexico. A number of oilseed mills in the Midwest and Southwest have processed small quantities of safflower seed during the past two decades.

Because of expansion in the production of safflower seed and the increased volume of oil available from this source, an investigation has been made at the Southern Laboratory to determine the suitability of the oil for food uses.

Although safflower-seed oil is generally classed as a drying oil suitable for use in paints and varnishes, it has also long been used as an edible oil in India and the Middle East. The Southern Laboratory wanted to find out whether oil from domestically grown safflower seed could be processed to yield edible oil and shortening comparable in quality with those produced from other oils.

An edible oil or salad oil of bland odor and flavor was obtained by laboratory processing of solvent-extracted safflower-seed oil, but it underwent undesirable flavor changes. It was very sensitive to light, and the freshly deodorized oil developed a taste suggesting rubber when exposed to daylight from a north window for as short a period as one-half hour.

Hardening of safflower-seed oil by hydrogenation to obtain a fat of shortening consistency improved the product with respect to undesirable flavor changes. However, the stability of the product to rancidity and flavor changes was not as good as that of similar products prepared from cottonseed or peanut oils. Further research may disclose ways for preventing the formation of undesirable flavors.

Tung Fruit Hulled on Farms

Tung farmers in the past have hesitated to use field hulling because they thought there would be a rapid break-down of the oil in the damaged nuts. However, studies by the Bureau's Tung Oil Laboratory at Bogalusa, La., show that field-hulled nuts can be safely stored and efficiently processed if dried to 10 percent moisture at drying temperatures below 165° F. Tung mills have installed well-designed seed dryers in which nuts hulled either in the field or at the mill can be successfully dried prior to storage and processing. Following the development of several types of field hullers, a number of tung growers adopted field hulling for the 1949 crop. An appreciable proportion of the crop of tung fruit was hulled with semiportable hullers which could be moved from orchard to orchard.

Field hulling reduces by half the weight of material hauled to the mill, leaves the hulls on the farm for use as mulch, and relieves the mill of the problem of hull disposal.

Cooperative studies at tung-oil mills reveal two primary causes for the loss of oil in the processing of tung fruit: (1) Some oil-rich kernel fragments are removed with hulls in the hulling operation; and (2) the presses are not capable of removing all the oil in the ground kernels. When the hulling is done at the mill, the loss in oil during the hulling operation is assumed by the mill, but when the fruit is hulled in the field, the grower stands any such losses. Subsequent losses resulting from incomplete extraction of the oil from the kernels are, of course, borne by the mill. At commercial mills about 85.5 percent of the oil in the whole (unhulled) fruit is normally recovered. When tung nuts that have been hulled in the field are delivered and processed, the oil recovery per unit weight should be higher because the hulls, which contain little oil, have already been removed. No information was available, however, on the yield of oil that should be obtained from field-hulled nuts. In view of the trend toward field hulling, information on hulling losses was needed so the tung grower could make an accurate comparison of the relative merits of selling hulled nuts or whole fruit to the mills.

For determining the loss of oil in hulling, on the basis of the whole fruit, the percentage of hulls in whole fruit was determined by hand shelling. Then the hull fraction obtained in commercial hulling was analyzed for its oil content. These two figures, multiplied, gave the percentage of oil lost as nut fragments in hulling.

With adequate analytical procedures available, the Bureau's Tung Oil Laboratory, in cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering, investigated commercial hulling of tung fruit in the field and at the mill. Samples of the whole fruit, hulls, and hulled nuts were collected during the hulling operations and analyzed at the Bogalusa laboratory. It was found that

the oil in the hulled nuts varied from 92.7 to 98.6 percent of that in the corresponding quantity of whole fruit, which meant that the loss of oil due to hulling was 1.4 to 7.3 percent of the total oil in the whole fruit. The condition and adjustment of the huller appeared to have much more influence than did the moisture content of the tung fruit on the efficiency of the hulling operation and on the percentage of shell removed from the tung nuts.

To determine whether or not it is advantageous to deliver hulled tung nuts rather than the whole fruit, the tung grower must consider not only the differential in price but also the reduction in gross weight as the result of hulling and the reduction in oil content. If a certain quantity of tung fruit contains 100 pounds of oil, the hulled nuts from this quantity of fruit will contain, on the average, 97.5 pounds of oil. If the mill can extract 85.5 percent of the total oil in the whole fruit, it would get 85.5 pounds of oil from the whole tung fruit but only 83.4 pounds of oil from the hulled nuts.

Study on Lipases Suggests How To Prevent Splitting of Fats and Oils

Fats and fatlike substances, known collectively as lipides, are very susceptible to enzymatic decomposition. When this occurs, free fatty acids are formed from fats. This explains the formation of free fatty acids in products rich in oil (like seeds) and not infrequently in the oil expressed therefrom. The destructive enzymes (lipases) occur in the seeds, and may also be formed by bacteria and molds. Thus lipases constitute a hazard in the storage of oilseeds and oils. Their action, however, has received only occasional attention. The principal reason is that no method was known for removing these fat-splitting enzymes from most of the agricultural commodities in which they occur, and thus it was not possible to study them in detail.

To cast light on this subject, the Enzyme Research Division first made a study of one plant that contains a great deal of lipase, namely the castor bean. The castor-bean enzyme appears to be typical of lipases in other oilseeds, but it is more stable and therefore can be extracted without decomposition.

Methods were devised for extracting the fat-splitting enzyme from castor beans and removing some of the impurities with little or no loss of activity. The enzyme was removed from the seed with the castor oil, and the oil was then removed from the mixture. A highly lipolytic substance, consisting mainly of protein, was obtained. Its activity was destroyed by water, unless some fat or other nonpolar substance was also present.

The effect of this enzyme on cottonseed oil was studied. When tested by the old established methods, which call for a little oil emulsified in a lot of water or buffer solution, the effect was small. But when tested under conditions more nearly like those existing in a tank of oil, where a lot of oil contains only a very little water, the effect was very great. As much as one-fifth of the fat could be split at room temperature to free fatty acids in a few hours, and the limit was reached when the water, not the enzyme or the fat, was used up. Free fatty acids and partly decomposed fats (monoglycerides) crystallized out of the mixture in a few days. Thus it is evident that very

decided alterations in the composition of a fat may result from the presence of even a little active lipase. Furthermore, the reaction goes very much faster than that of most enzymes at room temperature or below.

It was further observed that this energetic lipolytic reaction could be greatly inhibited by the presence of small amounts of fatlike substances that sometimes occur naturally in oils. For example, the castor-bean lipase was inhibited by palmitolinoleocephalin (synthetic and therefore of definitely known chemical composition) and by either hen-egg phosphatide or salmon-egg phosphatide but not by soybean phosphatide. This fact may explain why lipolysis is not a universal reaction in all crude oils, and it also points to the possibility of stopping this undesirable form of decomposition by the addition of appropriate chemicals.

Progress Made in Stabilizing Flavor of Soybean Oil

Soybean and cottonseed oils are the two most important vegetable oils of domestic origin. Cottonseed oil, both in liquid and solid (hydrogenated) form, has long been highly regarded for edible products. Soybean oil, derived from a crop of comparatively recent introduction into this country, serves both for protective coatings and for edible products. In using soybean oil for shortening and margarine, or as a salad oil, refiners and food producers at first attempted to apply without change the methods successfully developed and used for cottonseed oil. In the past few years great improvements have been made in the quality of soybean-oil products as a result of improved processing methods and concerted efforts of oilseed crushers, refiners, and food processors. When domestic soybean oil replaced most of the 1 billion pounds of foreign oil formerly imported annually but made unavailable by the war, further impetus was given to improvement of its quality for food uses. It became evident that one of the most serious disadvantages of the oil was its flavor instability. This flavor problem, long recognized by industry, is exceedingly difficult and complex. Many opinions and theories as to the cause and possible remedies have been expressed, but until recently very little data have been published.

The organizations of both growers and processors (American Soybean Association and National Soybean Processors Association) have cited the flavor instability of soybean oil as the most important problem for full utilization of this crop. Research on many phases of the problem has been under way, and a broad attack is being made under the leadership of the Northern Regional Research Laboratory. Additional impetus to the early work was given by use of Research and Marketing Act funds both at Peoria and, under contract, at the University of Pittsburgh.

The Germans reported that the beneficial effects of small amounts of citric acid added during deodorization of soybean oil resulted from the action of citric acid in removing traces of lecithin. The Northern Laboratory found, however, that the citric acid acted largely as a metal scavenger, removing or neutralizing the effects of small amounts of iron in the oil. Other equally effective metal scavengers have been found, and information on them has been published. An improved

spectrographic method recently developed permits the determination of the minute amounts of iron and copper that affect flavor stability of the oil. The presence of more than 0.1 p. p. m. (parts per million) of iron or 0.01 p. p. m. of copper definitely reduces the flavor stability of the oil after accelerated aging at 60° C.

It was found that cottonseed oil behaved otherwise. As much as 0.3 p. p. m. of iron added to cottonseed oil did not affect its flavor stability. Iron by itself, therefore, is not responsible for the instability. Soybean-oil glycerides contain a little linolenic acid, which is not one of the component acids of cottonseed-oil glycerides. Linolenic acid, one of the major components of linseed oil, is more reactive than most acids in edible vegetable oils. It has been guessed, without much evidence, that linolenic acid might be responsible for the undesirable flavors in aged soybean oil.

With Research and Marketing Act funds, more direct evidence on the role of linolenic acid has been obtained at the Northern Laboratory. The taste panel is able to distinguish clearly and identify aged soybean and cottonseed oils. Each has a characteristic flavor. By careful chemical research, and after several failures, a method was perfected for introducing linolenic acid into cottonseed oil. Chemically this is relatively simple, but to modify any oil chemically without introducing foreign flavors proved difficult. A modified cottonseed oil containing linolenic acid which was bland and essentially flavorless when fresh, was finally produced. A control sample of cottonseed oil, similarly treated but containing no linolenic acid, was also prepared. When the modified cottonseed oil, after aging, was submitted to the taste panel, with aged samples of soybean oil and unmodified cottonseed oil, the cottonseed oil containing linolenic acid was identified with statistical significance as soybean oil. The descriptions of its flavor, such as "fishy," "grassy," or "painty" were typical of those for aged soybean oil. This offers direct evidence that the presence of linolenic acid is at least partially responsible for the flavor instability of soybean oil.

This conclusion is supported qualitatively by the improved stability of a soybean-oil fraction obtained by extracting the original oil with furfural, a partially-miscible solvent that removes many minor constituents from the oil and markedly reduces the linolenic-acid content. A noticeably better soybean oil resulted when the linolenic acid was reduced from 7 to 9 percent to 2 to 4 percent.

The practical application of this new knowledge has not been neglected. In liquid oil, the linolenic acid content is not easily reduced, but control of the iron content or neutralization of its harmful effect may be possible. The Northern Laboratory found that the amount of iron naturally present in soybean oil is about 0.8 p. p. m., indicating that iron removal during processing is necessary for producing oil of highest food quality. Investigations were also made on the iron content of samples taken in the various stages of processing soybeans for oil by solvent extraction and refining the oil for food uses. These samples were obtained from the extraction tower, evaporators, and stripping columns and during alkali-refining and deodorization operations. The iron content of the oil rose during extraction, but was markedly reduced during alkali refining. In some of the cooperating soybean-oil plants, iron was reintroduced during deodoriza-

tion. The commercial samples, however, did not show any correlation between iron content and flavor stability, as measured by the taste panel. Some of the plants whose oils had a high iron content but still showed good stability, it was found, used metal deactivators during deodorization. The procedure of adding metal deactivators to soybean oil has been recommended to industry and was discussed briefly in the 1948 report. At the Northern Laboratory studies are under way on practical methods of treating and operating pilot-plant deodorizers to avoid introducing traces of iron during the final deodorization step. Comparison of laboratory deodorization in glass apparatus with commercial deodorization in metal equipment has emphasized the importance of this final step in its effect on the stability of the oil. Apparently there is still room for improvement in some commercial operations.

The chemical compounds actually responsible for the undesirable flavors in liquid and hardened soybean oils are of importance both in understanding the causes of instability and seeking remedies for it. These compounds are being studied at the University of Pittsburgh under a Research and Marketing Act contract. The amounts of strong-flavored compounds in soybean oil are extremely small, and it is certain that many are formed as the oil acquires "off" flavors. These odoriferous constituents can be removed from the oil by a steam distillation under reduced pressure, which is essentially the method used in commercial deodorization. A complex mixture is obtained, and the products from liquid oil may be different from those obtained from hardened oil.

Prior to the contract research, the University of Pittsburgh had found α -heptenal in the undesirable-flavor fractions from liquid soybean oil. Organoleptic tests both at that university and at the Northern Laboratory show that this volatile product contributes to the objectionable flavor. Work under the contract also established the presence of α -heptenal in the liquid oil itself. In addition, decadienal, acetaldehyde, and maleic dialdehyde have been identified in "heat-reverted" liquid soybean oil. The contract work has further shown that maleic dialdehyde, dipropyl ketone, and α -heptenal are present in the distillate from heat-reverted hardened oil. As each constituent is isolated and identified it or a derivative is compared with an authentic sample. As these compounds are found or synthesized in sufficient amounts, they are used in organoleptic tests to determine their flavor characteristics. Other compounds known to be present are under investigation.

Other vegetable oils have been compared with soybean oil by the Northern Laboratory's taste panel. Some of the oils may be of future importance or may become available in commercially significant quantities. The comparisons made, while of a preliminary nature, showed that fresh safflower and mustard oils are better than fresh sorghum and sunflower oils. None of these oils was outstanding in flavor stability. The evaluations were made on oils that had been alkali-refined, bleached, and deodorized in the laboratory by the methods used for soybean oil. Undoubtedly studies on any of these oils would suggest how to adapt the processing methods to the individual oils so as to obtain better products. Of this group of four oils, safflower oil may become available from domestic seed in commercial

quantities within the next few years. This crop is receiving a great deal of attention in western Nebraska, Colorado, and California as a potential oilseed. Safflower oil is excellent for use in protective coatings and may find its chief outlet in that field.

More Tank-Car Sludge Means Less Refining Loss for Soybean Oil

When tank cars of crude soybean oil are shipped from processor to refiner, a layer of sludge, or settlings, is frequently deposited in the bottom of the car. This material comes largely from the phosphatides and other nonglyceride components of the crude oil. Refiners dislike receiving tank cars containing sludge, mainly because of lower net weight of oil recoverable at destination and the increased difficulty of cleaning the tank. Present trading rules provide for price adjustments when the amount of sludge exceeds 150 pounds per tank car (about 61,000 pounds of oil). Because the price of oil is adjusted according to refining loss as measured on a sample taken when the tank car is loaded, the relationship between refining loss and sludge is important with respect to both the price of oil and the good will between buyer and seller. Tests to determine this relationship were undertaken by the Northern Laboratory at the request of the Technical Committee of the National Soybean Processors Association.

Preliminary tests, made under conditions simulating those prevailing in winter when sludge occurs most frequently in tank-car shipments of crude soybean oil, indicate that there is a decrease in refining loss as sludge accumulates and that the present penalties on both sludge and refining loss may need adjustment to be fair to both buyer and seller. Further tests are under way.

Composition of Soybean Phosphatides Determined

Soybean phosphatides are a byproduct of the soybean-processing industry. These compounds, often described as intermediate between fatty oils and proteins, are potentially available in quantities much greater than those now used by industry. At present, lecithin and other phosphatides are being used (but seldom in large amounts) as emulsifying agents in making food and industrial products such as candies, bread, textiles, tires, paints, printing inks, soaps, and dyes.

The actual composition of soybean phosphatides has been determined under a Research and Marketing Act project at the Northern Laboratory. Composition studies (with the Craig countercurrent distribution apparatus and immiscible solvents) showed three main groups of soybean phosphatides: Lecithins, 30 percent; cephalins, 30 percent; and phosphoinositides, 40 percent.

The choline-containing fraction (lecithin) and ethanolamine-containing fraction (cephalin) are soluble in alcohol; the remaining fraction consists of two complex phosphoinositides, one of which is soluble in alcohol and the other in hexane. Sugars usually accompany these substances, but uncertainty still exists as to whether they are merely carried along mechanically or are chemically combined. The sugars galactose and fructose have been identified, after hydrolysis, by paper chromatography. The isolation and identification of the

various components require a great deal of work; frequently it is necessary to modify an old method of analysis or devise a new one.

As the phosphatides from corn oil are reported to be superior for certain uses, where greater oxidative stability and increased stability of oil-phosphatide combinations are desired, a comparison was made of soybean and corn phosphatides. It was found that the same components are present in both, but in markedly different amounts. For example, the cephalin fraction of corn phosphatides amounts to 10 percent or less. Work is still under way, and further studies on the large-scale laboratory preparation of the phosphatide fractions are planned.

POTATOES AND POTATO PRODUCTS

Specific-Gravity Grading of Potatoes Has Limitations

Grading of potatoes on the basis of their specific gravity is being increasingly advocated as a method for selecting potatoes of uniform processing or cooking quality. Correlations between specific gravity and mealiness, and between mealiness and the tendency to disintegrate or slough have been observed repeatedly. Recent work at the Eastern Regional Research Laboratory indicates, however, that there are previously unrecognized limitations in this method of grading.

Potatoes of the same variety and lot, having uniform specific gravity, may vary greatly in their degree of sloughing, and presumably in their mealiness also. For example, sloughing decreased by 30 percent and 90 percent, respectively, when potatoes were stored for 7 weeks at 50° and 34° F., although the specific gravity remained essentially unchanged. After longer storage, the specific gravity of some of the potatoes increased as their weight decreased. This change was especially pronounced in potatoes stored at 75° F. Despite the increase in specific gravity, sloughing diminished.

It is apparent, therefore, that specific-gravity data alone are not sufficient for predetermining the quality of potatoes. Information on their history is also necessary, including such factors as temperature of storage and loss in weight.

In previous studies on the relation between specific gravity and mealiness whole potatoes were used. Because the specific gravity of tissues in any single potato varies greatly (from 1.070 to 1.110 or more in a potato having a mean gravity of 1.095), no exact relationship between gravity and mealiness could be established. In the recent experiments, however, 10-gram pieces of potato having practically uniform density were used. It was thus possible to study the true relationship between specific gravity and sloughing, because pieces having the same density as those used in the initial experiments could be selected from potatoes whose density had changed. The specific-gravity measurements indicated rather accurately the starch content of all potatoes, excepting those subjected to cold (34° F.) storage.

An explanation for the differences in sloughing and mealiness in potatoes may be based on differences in the swelling capacity of starch and in the concentration of starch per cell. When potato tissues are heated, the starch granules within the cells gelatinize, swell, and distend the cell walls. This results in weakening, or complete failure, of

the intercellular cement. In the case of complete failure, single cells or clusters of cells slough from the main body of tissue, and the tissue becomes excessively mealy. Factors that decrease the swelling of starch (for example, treatment with calcium salts, increase in sucrose or salt concentration, or decrease in water content during storage) inhibit sloughing.

The concentration of starch per cell likewise affects to a marked extent the texture of potato tissues. In freshly harvested potatoes, the concentration of starch per cell is at a maximum, and the highest degree of sloughing occurs. During storage, however, the concentration of starch per cell decreases, because some of the starch is consumed in respiration or converted to sugar, whereas the number of cells in the potato remains constant. The cells may shrink in volume, which effectively increases the thickness of their walls. Although specific-gravity measurements of whole potatoes may reveal their starch content, they in no way indicate the concentration of starch per cell. Accordingly, potatoes that increase in specific gravity and in percentage of starch during storage may actually decline in mealiness, owing to a decrease in the starch content of the individual cells.

Some Surplus Potatoes Profitably Converted to Duck Feed

In the course of the Eastern Laboratory's work on developing cheap methods for producing potato flour under an RMA project, methods for making feeds were also devised. One of the methods entails grinding the raw potatoes in a hammer mill and drying them in a steam-tube drier while continuously recycling a portion of the dried product to avoid sticking. The dried product is ground to a size suitable for incorporation in feed. The product can be made for \$24 a ton, exclusive of the cost of the raw potatoes and selling costs.

Because the quantity of cull potatoes on Long Island alone may amount to as much as 3 million bushels in a typical year and ducks are raised in the same area in large numbers, arrangements were made with the New York State Agricultural Experiment Station and the Bureau of Animal Industry to determine the value of dried raw potatoes in duck rations. It had been previously reported that, although cooked potatoes could be used in duck feeding, dried raw potatoes were unsuitable. Feeding tests, however, showed this to be untrue. They showed that dried raw potatoes could be substituted for 20 percent of the corn meal or wheat standard middlings, or for 10 percent of corn meal plus 10 percent of wheat standard middlings, in the mixed feed with no effect on the live weight of ducks at 9 weeks of age. Out of 2,000 ducklings started in the experiment, 1,950 were marketable at its end.

With corn meal and wheat middlings selling at 3.45 cents a pound in New York, a potato grower, if also producing duck feed, could pay the cost of manufacture and realize 51 cents per 100 pounds of culls by selling the potato feed at the current price of corn meal and wheat middlings, or he could charge in his culls at no cost and sell his product at more than twice its cost to manufacture. These figures are based on a potato-drying plant costing about \$80,000 and processing 75 tons of potatoes into 17.3 tons of feed every 24 hours.

Current studies indicate that potatoes may be dried at least as cheaply in a rotary alfalfa drier. Unlike the steam-tube drier, this type of drier would not require a boiler house. Although no duck feeding tests have been made on the product from the alfalfa drier, the results should be about the same as with the product from a steam-tube drier, the compositions of the two products being similar.

GRAIN CROPS AND RESIDUES

Ratio of Crude Fat to Crude Fiber Indicates Milling Character of Wheat

The milling characteristics of wheats differ with variety, crop year (weather), and locality where grown. Poor-milling wheats give low yields of flour and decrease mill capacity. Wheat varieties having undesirable milling properties, however, may be those best suited agronomically to certain areas. Consequently farmers in those areas must choose between growing a poor-milling wheat that is difficult to market or be satisfied with less productive varieties. The development of new varieties, having both good milling and good agronomic properties, could be speeded up if poor-milling strains could be detected and discarded at an early stage in breeding experiments; but at present new strains must be propagated over several years to provide enough grain for milling tests. Exploratory work begun at the Western Regional Research Laboratory suggests that differences in the composition of wheats influence milling behavior in previously unrecognized ways, and that laboratory methods, applicable to small samples, could be developed for the detection of poor-milling hybrids.

Rex, a variety adapted to production in the Pacific Northwest, has poor milling characteristics; the separation of bran from the floury portion of the kernel is difficult, and the flour tends to clog sifters. These properties may be transmitted to hybrids when Rex is used as a parent in wheat breeding. In cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering, which supplied samples and provided experimental milling data, the Western Laboratory sought the characteristics of composition or structure of Rex wheat that could account for its poor milling behavior.

Other investigators have reported that the sifting behavior of flour is governed by the tendency of its particles to clump rather than by the size of the particles. In both the grain and the flour, the Western Laboratory found that Rex samples consistently contained more crude fat than samples of other varieties. As Rex flour from which fat had been extracted sifted readily, it seemed possible that the relatively high fat content of Rex wheat contributes to the difficulty of sifting Rex flour by causing particles to clump.

Limited microscopic examinations of kernel sections of Rex and Elgin (a variety having good milling characteristics) wheats showed that in Rex one of the intermediate bran layers was thicker. The Rex sample also contained slightly less crude fiber than the Elgin sample. As nearly all of the crude fiber of wheat is contained in the bran, the crude-fiber content of the whole wheat might indicate roughly the relative toughness of the bran. Bran lacking sufficient toughness is

difficult to separate from the flour-producing portions of the wheat kernel by present milling methods.

On the basis of such considerations, the ratio of crude fat to crude fiber was determined for samples representing different varieties, localities, and crop years. The values for 12 Rex-wheat samples and for 10 Rex hybrids of poor milling properties were consistently higher than those for 39 samples of other varieties (Elgin, Hymore, Golden, Triplet, Boart, Brevas, and Orfed) and 1 Rex hybrid, all of which were considered good or intermediate in milling characteristics. Two varieties (Federation and Marfed) gave ratios intermediate and somewhat overlapping the two groups mentioned previously. Marfed occasionally has been found difficult to mill on a commercial scale.

The crude fat-to-crude fiber ratio thus offers a promising means of detecting Rexlike milling characteristics on small samples, as only 6 grams of wheat is sufficient for the required analyses, as compared with 5 pounds for conventional milling tests. Furthermore, if specific features of composition and structure can be related to milling behavior, as this work indicates, further investigation may provide more precise methods and may suggest treatment to improve the milling properties of wheats.

Corrugated Strawboard Now Produced Has Superior Quality

The main use for straw in the paper and board industry of the United States is in the manufacture of corrugated board for packaging.

Until about 1948, corrugated strawboard was inferior, particularly in rigidity, to board made from pulpwood by either the semichemical sulfate process or the neutral sulfite process. A research program in cooperation with strawboard companies was then initiated by the Northern Laboratory to improve the quality and uniformity of corrugated strawboard and to improve pulping and refining processes. This research program, which was expedited by technicians supplied under cooperative agreements by three industrial companies, has been very successful. Most of the strawboard mills have applied the findings, with the result that strawboard now usually excels competitive wood products in stiffness.

The first step in the research program involved tests on strawboard pulp, taken from the beaters and the head boxes of the paper machines, and tests on samples of paper being made concurrently on the machines. On the basis of these tests, improved refining procedures were developed whereby the cooked pulp could be processed in such a way as to develop its full strength on the paper machine.

The next step was a thorough investigation of cooking procedures adapted for use in the usual rotary digesters of strawboard mills. These studies, involving about 85 cooks, showed that the use of lime, which has been the sole or major chemical cooking agent for straw during the past 100 years, produced pulp inferior in quality to that produced by cooking with other chemicals. Each of four chemical cooking procedures (using caustic soda, neutral sodium sulfite, caustic soda and neutral sulfite, or neutral sulfite and soda ash) produced pulps capable of making stiffer and higher-quality strawboard than was possible under previous mill practice. Because of the differences in equipment and facilities of various mills it was highly desirable

that several improved cooking methods be available, so that each mill might select the modified procedure best suited to its own needs. Comparative tests on recently produced pulpwood board and strawboard confirmed the marked improvement made in commercial corrugated strawboard.

Investigations on cooking procedures were continued with emphasis on the use of the mechano-chemical process, which was announced by the Laboratory last year, and on better pulp-refining operations. This simplified, economical pulping process, which utilizes the type of machine that is ordinarily used for pulping wastepaper and involves simultaneous mechanical and chemical action, eliminates the need for pressure-cooking equipment and greatly reduces the time required for pulping. The operation of the mechano-chemical process in the manufacture of corrugated strawboard has been successfully demonstrated on a commercial scale in one strawboard mill by cooking straw with either 6 percent lime and 4 percent soda ash, or with 5 percent caustic soda (on the basis of dry straw) for a total cooking period of 1 hour. The cooking cycle, from the filling of the pulper to dumping, was 90 minutes. It was not necessary to chop or dust the straw prior to cooking. The bale wires were simply removed, and the straw was then quickly dumped into the pulper containing the hot cooking liquor. A very satisfactory pulp was obtained.

Cobs From Hybrid Corn Contain More Pentosans

Results of a study on the composition of corncobs, at the Northern Laboratory, show that the pentosan content of cobs from hybrid corn is higher than that of cobs from open-pollinated varieties. The pentosan content (determined as furfural) of open-pollinated varieties was 20.4 percent or less; that of most hybrids was about 23.4 percent. The cobs of one hybrid variety yielded 26.3 percent of furfural. The lignin content of most hybrids is around 12 percent, compared with 18 to 20 percent for open-pollinated varieties. However, one hybrid contained about 23 percent of lignin. This investigation is not yet completed, but the results indicate the possibility of developing corncobs in which pentosan, lignin, or cellulose predominates. High pentosan content is extremely important if cobs are to be used for furfural manufacture.

Use of Ground Cobs and Nutshells for Air-Blast Cleaning Increases

Use of ground corncobs, pecan and walnut shells, and apricot and peach pits as grit in air-blast cleaning, which was pioneered by the Northern Laboratory, is increasing. A large automobile manufacturer has distributed information on corncob grits among his agencies for the purpose of creating interest in and facilitating the utilization of this material for cleaning engines and other metallic parts. One of our Air Force bases has distributed similar information on the use of pecan and black-walnut shell grits.

A new use for corncob grits is in finishing parts for the hydramatic and similar automatic driving mechanisms. The newest and largest potential use, however, is in low-pressure blasting to clean electric motors and generators, supplanting a solvent process which was time-

consuming, costly, and hazardous. In the new method the grits are blown under about 30 pounds air pressure on the motors and generators to degraze them.

Other possible applications of air blasting with such organic grits are in cleaning machine tools, plate glass, painted surfaces, railway tank cars, holds of seagoing tankers, and brick and stone facings on buildings.

A large manufacturer is now marketing an air-blast unit designed specifically for using grits produced from agricultural residues.

Liquid Motor Fuels Made Experimentally From Farm Residues

Investigations on the experimental production of alcohol and other liquid motor fuels from corncobs and other farm residues, under the Synthetic Liquid Fuels project at the Northern Laboratory, were discussed in the 1947 report.

Continued studies show that the basic principle of separately hydrolyzing the hemicelluloses and the cellulose in crop residues is applicable for large-scale plant operation. In the first step of the process, namely, the hydrolysis of the pentosans to pentose with dilute sulfuric acid, routine operation on a semiworks scale has given yields up to 75 percent of theoretical. Progress made in the development of the second step, i. e., hydrolysis of the cellulose to dextrose with concentrated sulfuric acid, indicates that satisfactory results can also be achieved in this phase.

More recently, large-scale laboratory experiments have demonstrated that the rate of conversion of the pentosans to pentose can be increased by 15 percent when the continuous countercurrent hydrolyzation at atmospheric pressure, which has been used heretofore, is replaced by continuous concurrent hydrolyzation under pressure. The yield of pentose is greater by the latter method, because of a very marked decrease in the quantity of unattacked pentosans in the residues. Through this change, the raw-material cost per pound of pentose in the hydrolyzate can be lowered by one-third.

Investigations on the fermentation of pentosan-hydrolyzate liquors in the pilot plant at the Northern Laboratory indicate that satisfactory yields of butanol, acetone, and ethanol can be obtained when the butanol hydrolyzate and 25 percent corn. They show further that, in addition to other factors, when the copper content of the hydrolyzates is above certain levels, the fermentation of the sugars is very difficult. Copper results from the reaction of the hydrolyzing acid on the alloys from which the processing equipment is made. To eliminate the detrimental influence of the copper, a simple and economical procedure was developed for removing copper to a point where it is negligible. The procedure consists of passing the hot hydrolyzate through a column packed with scrap iron, whereupon copper separates as metal and an equivalent quantity of iron is dissolved. The hydrolyzates, thus treated, have given greatly improved fermentation results.

A limited number of samples of pressure-cooked pentosan hydrolyzates prepared in bronze cookers on a laboratory scale have been

tested for fermentability. The results indicate that fermentation characteristics of pressure-cooked hydrolyzates are different from those of pentosan hydrolyzates cooked at atmospheric pressure. Further experiments are expected to show how to ferment the pressure-cooked hydrolyzates as satisfactorily as the others.

The Synthetic Liquid Fuels project was terminated at the end of the fiscal year because of expiration of the time during which Public Law 290 of April 5, 1944, authorized studies on the production of synthetic liquid fuels from nonpetroleum sources.

STARCH AND DERIVATIVES

Fundamental Studies Made on Structure of Starch Granule

Industrial processing of grains is based on the physical and chemical properties of the kernel constituents. From knowledge of the behavior of starch while absorbing water, a better understanding of the many problems in the processing of this major constituent of grains is being sought at the Northern Laboratory. The variation of water-sorption capacity as a function of physical treatment and chemical modification of cornstarch and other starches has been measured; also the relationship between water sorption and the factors of surface area, specific gravity, and degree of swelling. Some measurements are still in progress, but results obtained thus far emphasize the importance of the starch-granule structure in determining the properties of starch.

As shown by surface-area measurements of starches at low temperatures with gas-adsorption techniques, starch granules are not permeable to nitrogen. On the other hand, water vapor is readily absorbed, and each type of starch exhibits, upon water sorption, a characteristic swelling. Tapioca starch and waxy-maize starch, which have similar uses, show similar swelling, for the amount of water absorbed, and differ from corn and potato starches by exhibiting greater swelling. The course of the swelling during water absorption is interpreted as being controlled by the unique organization or physical arrangement of the structural parts of each type of starch. The similarity between the swelling characteristics of tapioca starch and those of waxy-maize starch shows the importance of large-scale organization or arrangement in obscuring molecular differences such as amylose-amylopectin ratio. It was also found that water-sorption capacity of starches is usually increased in the initial phase of chemical modification. This has been attributed to loosened granule structure, despite the absence of granule damage at this stage.

Esterification has a complex effect on starch-granule structure and water sorption. Loss of hydrophilic hydroxyl groups by esterification reduces water sorption, whereas opening of the granule structure by introduction of the ester molecules increases it. Ester linkages themselves may show a limited sorptive capacity. The observed water sorption is interpreted as the resultant of these effects.

Fundamental studies on starch-granule structure will be continued. The data will be interpreted in terms of pertinent physical-chemical theory to provide a fundamental basis for developing new uses for starch, where possible. They will be used also to explain the physical

properties of commercial starches and the differences between starches from various sources.

Starch Sponge Useful as Hemostatic Agent

Medical workers and pharmaceutical companies are interested in starch sponge as a means of controlling hemorrhage. Recent publication by the medical profession of the clinical findings on successful application of starch sponge as a hemostatic agent in difficult operative cases has stimulated study of the product.

The Northern Laboratory's work on a process for making starch sponge was described in the 1945 report. Briefly, its preparation and properties are as follows. A starch paste, containing about 7 percent of cooked starch, is slowly frozen at temperatures between -12° and -18° C. When the frozen mass is thawed, it retains the shape of the container upon removal. The water present in the original paste remains absorbed in the porous material, but by pressing the spongelike substance, most of the water can be expressed. The pressed wet sponge is soft and resilient and, upon release of the pressure, immediately resumes its original size and shape. It will readily reabsorb all the water that was pressed from it. If the sponge is dried, it shrinks slightly and becomes a hard, somewhat brittle, white porous solid. When brought in contact with water, the dried sponge quickly absorbs about 16 times its weight of water and swells to its original size. The dried sponge can be ground to a fine, absorbent powder.

Different methods of using starch sponge for medical purposes have been tested clinically. The Northern Laboratory developed a method for preparing starch sponge on bandages of surgical gauze. Several such bandages were used successfully in obstetrical cases to check hemorrhage following Caesarean operations. Blocks of starch sponge also have been used for the same purpose in natural childbirth and in a number of surgical cases with equally favorable results. Special interest has been shown in the possibility of using starch-sponge blocks in abdominal surgery. Removal of the starch sponge after operations would not be necessary because the body enzymes break down the starch to simple sugars that can be absorbed and used by the body.

Powdered starch sponge also has excellent hemostatic properties. Its use on gauze in genito-urinary operations has brought reports that blood loss was reduced 70 percent. The starch-sponge-on-gauze bandage can be removed with ease and without pain to the patient; removal of ordinary dry-gauze bandages causes pain and frequently starts hemorrhage. It is reported that a number of cases of gastric hemorrhage have been treated by oral administration of powdered starch sponge, and in every case hemorrhage ceased, apparently as the result of the treatment. The plastic-surgery division of a large university is experimenting with the starch-sponge powder as a means for granulating surfaces prior to skin grafting.

Dissemination of information on production, use, and value of starch sponge to the leading pharmaceutical firms in the country has increased practical studies on the product. Active clinical studies by several drug companies are expected to lead to early commercial

production of starch sponge in quantities that may require more than 1 million pounds of starch annually. The willingness of members of the pharmaceutical industry to engage in practical confirmatory tests is expected to shorten the time required to bring the product into wide use.

Addition of Calcium Carbonate to Starch Reduces Explosion Hazard in Candy Molding

Information that promises to eliminate the danger of explosions in candy-manufacturing plants has been obtained in joint research by the Agricultural Chemical Research Division and the National Confectioners Association.

In the past, candy makers generally have used starch as a casting medium for marshmallows and other candies that require molding. Starch is entirely satisfactory for this purpose, as far as its effects on the candy are concerned, but, like many other finely powdered organic materials, its mixtures with air are explosive.

During research to increase the utilization of agricultural products in confectionery, chemists of the New Orleans laboratory and the candy maker stationed there by the National Confectioners Association found that an inorganic material that will not explode can be mixed with the starch with no ill effects on the candy produced. This material is U.S.P. food-grade calcium carbonate, a specially precipitated form of the substance of which chalk is made.

In tests made by a commercial laboratory, the pressure developed by the explosion of pure starch in laboratory test bombs was about 75 pounds per square inch. This was reduced to about half as much for a mixture containing a little more than 20 percent calcium carbonate, while 55 percent calcium carbonate in the starch mixture reduced the pressure to practically zero.

It was found that mixtures of starch with 25 to 75 percent calcium carbonate were as satisfactory as starch alone for the casting of marshmallow and jelly-type candies, as well as of low-moisture hard creams. The experiments were made with laboratory-scale batches, single trays, having impressions made by hand, being used for each mixture and type of candy.

Several manufacturers later tested the use of calcium carbonate with starch in full-scale, continuous operations. Although some difficulties were encountered, they are not insurmountable and are offset by the advantages gained, according to the companies. One firm reported that calcium carbonate added to its molding starch, and properly used, gave sufficiently better results in casting marshmallows to warrant its adoption, apart from consideration of safety.

Several insurance companies have inquired about these investigations and the industrial application of the new molding material. Once its safety is fully established, the savings in insurance on plants adopting it may be considerable.

Further investigations have shown that calcium carbonate has a definite place in the manufacture of some candies which do not require molding. In stick candy, where a high gloss finish is not required, it is useful to prevent stickiness and the running of stripes. Its effectiveness in promoting the rapid, smooth graining of fudge also has been

established, and recent experiments show that it can hasten the grain-
ing of pulled grained mint candies.

FERMENTATION AGENTS AND PRODUCTS

Alcohol Plant Proves Value of Mold Amylase as Malt Substitute

A method for producing mold amylase, developed by the Northern Laboratory for use as a starch-converting enzyme in the production of alcohol from grain, was discussed in the 1948 report. Experiments on a pilot-plant scale had shown that this fungal amylase, obtained from submerged cultures of *Aspergillus niger*, could be used to replace malt entirely with no decrease in yield of alcohol. Furthermore, a potential saving in manufacturing cost of 3 to 5 cents per wine gallon of alcohol produced seemed possible through the use of the method.

Although interested in the Laboratory's mold-amylase process, the alcohol industry was reluctant to use the new process on a plant scale for three reasons: (1) Little was known about the effect of mold amylase on certain factors that influence quality of the resulting alcohol and the byproduct feed; (2) more assurance was needed that it is feasible to conduct pure-culture fermentations on a large scale in an alcohol plant; and (3) it was necessary to know either that regular plant equipment could be employed with mold amylase or that only minor alterations would be required to adapt a distillery for operation with the new conversion agent.

To supply the necessary information, the Grain Processing Corporation of Muscatine, Iowa, under a Research and Marketing Act contract, has conducted full-scale alcohol fermentations in its plant, using mold amylase in place of malt for the conversion of starch to sugar in mashes prepared from grains. Four successful runs were completed at the Muscatine plant, each run involving the preparation of about 25,000 gallons of mold-amylase liquor, of specified quality, and the employment of this liquor as the sole conversion agent in the production of alcohol from 5,000 bushels or more of a good grade of corn. The yields of alcohol equalled or slightly exceeded those obtained when malt was used. Processing operations in the distillery were normal during the runs.

Further pilot-plant studies at the Northern Laboratory are being directed toward the preparation and use of mold amylase to produce alcohol from damaged corn and from sound and damaged grains other than corn. Moldy corn, sound wheat of both hard and soft types, and sound grain sorghum have already been investigated. The enzymatic liquor used in each case was prepared from thin distillery slop obtained from the kind of grain under test. To this slop, which contained 4 percent solids, 5 percent of the meal of that same grain was added. For all grains tested thus far, alcohol yields have been at least equal to those obtained by the use of malt as the converting agent. No operating difficulties have been encountered in any of the tests. Studies in the pilot plant on the applicability of mold amylase to the conversion of other sound and unsound grains are under way.

A medium consisting of starch and inorganic salts was developed in the laboratory for the satisfactory production of mold amylase in submerged, aerated cultures of *Aspergillus niger* NRRL 330. Yields

of more than 30 maltase units per milliliter of culture have been obtained. The filtrate from the medium used for maltase production was concentrated under vacuum at 38° C. to about one-tenth of its original volume without loss of enzymatic potency. Dry preparations having a potency of 50,000 maltase units per gram were obtained by further purification of the liquid concentrate, involving precipitation with ethanol and ammonium sulfate and dialysis. *Aspergillus niger* NRRL 330 produces enzymes that are capable of hydrolyzing isomaltose to a greater degree than are filtrates from *Aspergillus niger* NRRL 337, reported on previously. This ability to break down isomaltose is of interest in starch chemistry, because the linkage between the two glucose units in the isomaltose molecule is of the alpha-1,6 type. This type of linkage occurs at the branching points of the amylopectin molecule and is resistant to the action of the common amylolytic enzymes such as maltase.

Studies Made on Macerans Amylase

Besides the breakdown of starch to dextrans and sugars that is caused by the action of ordinary amylases (such as those found in malt and sweetpotatoes), an enzymatic breakdown of starch that produces entirely different end products is known. This breakdown is caused by macerans amylase, which is excreted by a certain bacillus (*Aerobacillus macerans*). It gives rise to dextrans that crystallize readily and have no reducing properties (Scharinger dextrans). Such dextrans cannot be fermented to alcohol by ordinary yeast. The organism that produces macerans amylase is of frequent occurrence in water, soil, and air. Its presence may be a hazard in the handling of starchy materials intended for fermentation, because its amylase changes starch to unfermentable substances.

An investigation of the amylase of *Bacillus macerans* has been made by the Enzyme Research Division under a fellowship of the Corn Industries Research Foundation. It required the development of a quick and quantitative method for determining how much enzyme is present in a given batch, some improvement in the methods of growing the cultures in order to obtain quantities of the enzyme for study, and discovery of ways to concentrate and purify the enzyme when it has been excreted by the organism.

A quick and accurate method for the assay of the enzyme in culture fluids and concentrates has been devised. It is based on the fact that after starch is digested by macerans amylase the product no longer gives a blue color with iodine.

The enzyme is now being produced satisfactorily on a laboratory scale. It is concentrated by evaporating the culture fluid in vacuum and then precipitating with ammonium sulfate. It is purified by adsorption on starch from dilute alcoholic solutions and elution from the starch with water. An 8- to 10-fold increase in purity has thus been obtained, but the preparation is still not the pure protein. Such preparations are potent, however, for under suitable conditions they can digest more than 4,000 times their own weight of starch in 10 minutes.

Studies have been made of the action of the purified enzyme, to be repeated when final purification is achieved. It was found that

dextrins produced by the usual amylases can be digested as well as starch by macerans amylase. Thus the two types of starch breakdown may proceed together. The presence of certain sugars (maltose, glucose, and cellobiose) accelerates the action of macerans amylase on starch, but it is not certain that the same end products are formed as when these sugars are absent.

In the absence of sugars, yields of Schardinger dextrins varying from 50 to 70 percent of the weight of starch taken have been obtained. This is much higher than can be gotten by using the living organisms, because they decompose starch in other ways as well as by forming Schardinger dextrins.

Alcohol in Motor Fuel Reduces Cylinder Wear

The efficient and practical use of alcohol in combination with gasoline as a motor fuel for internal-combustion engines requires investigation of a number of specific, closely interrelated problems of a technical nature. These problems are being studied at the Northern Laboratory, some of them under an RMA project, through the application of two methods of alcohol utilization in gasoline engines which were discussed in last year's report. In one method, anhydrous, or water-free, alcohol is blended with gasoline for use as engine fuel; in the other, a mixture of alcohol and water is used to supplement the gasoline fuel by being injected, intermittently and in regulated quantity, into the intake manifold of the engine while it operates on straight gasoline as the basic fuel. Many of the fundamental data obtained for either method are applicable to the other.

The effects, during engine operation, of alcohol-gasoline blends and of alcohol-water mixtures (in combination with gasoline) on cylinder wear and carbon deposits are among the specific problems to be solved. The scope of investigation is substantially widened, however, because, as required by law, the alcohol must be denatured before use. The introduction of any one of the accepted denaturants might be expected to modify at least some of the effects shown by undenatured alcohol. The behavior of various denaturants in causing separation of the components of engine fuel at low temperature and in promoting gum formation presents further problems of a very practical nature.

To compare cylinder wear and carbon deposits, a series of cylinder-wear tests were conducted in a standard-type automobile engine, on a stationary mount, with straight gasoline and with a 25:75 percent blend of denatured alcohol with gasoline. The tests were based on running time of 200 hours. The engine was operated automatically through consecutive 15-minute cycles until 200 hours of actual engine-operating time had accumulated. Each cycle consisted of a 3-minute period of idling, a 7-minute period of operation under road load, and finally a 5-minute period of cooling the jacket and crankcase oil to specific temperatures.

Measurements of cylinder-wall wear were made at a series of points at each of five levels of the cylinder. Wear was determined from the change in depth of a pointed indentation made in the cylinder wall. Depth changes were calculated from the reduction in dimensions of the surface openings of the indentations measured with a special microscope attachment before and after each run. The average of

measurements taken at all points in the cylinder wall showed 0.0005 inch of wear for straight gasoline and 0.0003 inch of wear for the 25:75 percent blend of denatured alcohol and gasoline. These results are evidence that the denatured alcohol blend, with the particular denaturant used, produced less wear than did the straight gasoline. No harmful effect of the alcohol denaturant was observed in the engine.

Inspection after each test of the carbon deposits formed showed that deposits resulting from use of the blended fuel were fluffy, whereas those from straight gasoline were hard.

Test runs to determine the effects of alcohol and denaturants on cylinder deposits were made also in another standard-type automobile engine. This engine was operated steadily at road load for about 250 hours of running time (equivalent to about a 10,000-mile run) with no idling or cooling periods. The fuels used were the same as those used in the 200-hour test. The carbon deposits formed with the blended fuel again were observed to be more fluffy than those from straight gasoline. As in the first test, no evidence of detrimental effects from the use of the denaturant was noted. As a matter of fact, more favorable results were obtained in this respect with the alcohol fuel.

Selection of a suitable alcohol denaturant from among those permitted by the Alcohol Tax Unit depends upon other factors, as well as its effect on the engine. The cost, of course, must not be prohibitive. The stability of the denaturant in various alcohol-water mixtures over a given range must be determined. A number of alcohol denaturants were tested, at temperatures down to -30° F., for their ability to stay in solution in alcohol-water mixtures and to cause no component separation in the mixtures. Corrosiveness of the denaturants was also tested. Certain of these denaturants were then screened by the performance of fuels containing them on a Lauson-type, one-cylinder, stationary engine before a selection was made of a denaturant for the 200-hour full-scale automotive engine tests.

Research is continuing on engine wear and carbon deposits and on complete characterization of other alcohol denaturants which would be suitable for use with alcohol motor fuels. Other important phases of research on the utilization of alcohol as a motor fuel, conducted as Research and Marketing Act projects, include the Laboratory's contracts with the Department of the Army, for studying the performance of alcohol-water injection under truck-fleet operation and under low-temperature operation, and with the Armour Research Foundation, Chicago, Ill., for determining the effect of alcohol-water injection on various detergent-type lubricating oils.

SUGARS AND SUGAR PRODUCTS •

Protective Coating Material Made From Sugar

A new product having industrial possibilities as a protective coating has been made from table sugar by the Eastern Regional Research Laboratory in cooperation with the Sugar Research Foundation, Inc. This product, known as allyl sucrose, is prepared from sucrose (cane or beet sugar) and allyl chloride in the presence of caustic soda. Allyl chloride is an inexpensive industrial chemical made from propylene—a byproduct of the petroleum industry. Chemically, allyl

sucrose is an allyl ether of sucrose. Although it is possible to substitute eight hydrogen atoms in each molecule of sucrose with allyl groups, it is practicable to substitute only about six hydrogen atoms with allyl groups.

Allyl sucrose is soluble in various organic solvents, including alcohol, acetone, turpentine, benzene, toluene, and xylene. It is an extremely reactive compound and has a tendency to polymerize, that is, to form large inert molecules by the combination of many small chemically active molecules. As the solid polymerized product is insoluble in most organic solvents, it is suitable for use in protective coatings.

In practice, allyl sucrose is partly polymerized by passing air or oxygen through it at elevated temperatures before dissolving it in a suitable solvent. The solution is then applied by spraying or brushing. Allyl sucrose is compatible with drying oils, and, when added to drying or semidrying oils, it decreases the time required for drying. This is advantageous in coatings and other industrial applications.

Liquid allyl sucrose may be used as such, or in combination with other coating materials in organic solvents, with or without plasticizers. Special formulations are required when resistance to water or aqueous solutions is desired. For example, a copolymer of allyl sucrose and styrene, cured at high temperature, has good resistance to water.

Allyl sucrose has been produced at the Laboratory on a pilot-plant scale, and samples have been supplied to industries for evaluation in various applications.

Saccharates Made From Corn Sugar for New Industrial Use

The discovery that potassium acid saccharate prevents the precipitation of lime in hard-water solutions containing 2 percent of sodium hydroxide induced the Northern Laboratory to carry out pilot-plant experiments on the process by which it had previously made saccharic acid, essential for the preparation of potassium acid saccharate and other saccharates.

In this process, which was mentioned in the report for 1947, dextrose (corn sugar) is oxidized with nitric acid to form saccharic acid in solution. Neutralization of the acidic solution with potassium hydroxide yields potassium saccharate, and partial neutralization yields the acid saccharate. The first pilot-plant preparations of potassium acid saccharate were not economical, because the large excess of nitric acid required to oxidize the dextrose and the need for a correspondingly large amount of potassium hydroxide for neutralization made the chemicals too costly. Difficulty was also experienced in getting a white product, because color was formed at the high temperatures created by the neutralization reaction.

Newly developed methods have reduced the cost of chemicals required in the pilot-plant production of potassium acid saccharate and, at the same time, have improved the quality of the product. The nitric-acid requirement has been lowered to such an extent that dextrose can be oxidized with as little as $2\frac{1}{2}$ moles of nitric acid to 1 mole of the sugar. In a single-batch experiment, a 3 to 1 molal ratio yielded 50 pounds of potassium acid saccharate from 100 pounds of

dextrose, representing a satisfactory yield for this type of reaction. A further reduction in the cost of chemicals has been attained in the neutralization operation. The oxidation liquor is neutralized in two steps. In the first, about 60 percent of the potassium acid saccharate is recovered by reducing the pH to 3.8 with potassium hydroxide. In the second, the liquor is adjusted to pH 9 with potassium hydroxide and then reacidified to pH 3.4, after which the remainder of the acid salt can be recovered. Temperatures are held low during these neutralizations in order to obtain white crystals of the salt and light-colored solutions. It was found that a reduction in processing and standing time also aids in holding color formation to a minimum.

Examination of the liquor remaining after removal of the potassium acid saccharate showed that it, like the salt, prevents precipitation of lime. Neutralization of the oxidation liquor with the cheaper sodium hydroxide also yielded a liquid product having the same effect on lime salts. This suggested that a cheap agent of this kind can be produced from hydrol, a byproduct of dextrose manufacture from cornstarch, by oxidation with a small amount of nitric acid (1 to 1 molal ratio), followed by neutralization with sodium hydroxide. These inexpensive products have potential value in hard-water areas for large-scale cleaning tasks, such as those in the bottling industries.

Synthetic Detergents Made From Dextrose

Novel detergent products are being made from two types of substances, fatty acids and dextrose, that are derived from agricultural raw materials. Studies at the Northern Laboratory on the chemical reactions of dextrose to develop new industrially important compounds from this relatively low-cost material have resulted in a number of derivatives which are excellent foaming agents in water. Gluconic acid, derived from the sugar known both as glucose and dextrose, was employed as the starting compound. This new group of detergents, consisting of a series of sulfated gluconamides, was synthesized by converting gluconic acid to glucono-lactone, condensing this with fatty acid amines, and then sulfating with chlorosulfonic acid.

The soil-removing efficiency of the new synthetic detergents has been determined by the Bureau of Human Nutrition and Home Economics of this Department. The results of the tests showed that, as the length of the alkyl chain in the detergent increases, the detergent improves in soil-removing ability. The N-tetradecyl- and N-hexadecyl-D-gluconamide sodium sesquisulfates and N-octadecyl-D-gluconamide sodium disulfate have excellent surface-active and wetting properties. The hexadecyl and octadecyl derivatives remove about as much soil as does soap in distilled water. On the other hand, in water of 300 p. p. m. hardness and at concentrations of 0.35 percent and below, all the gluconamide detergents were more effective than the standard soap.

Preliminary tests showed that the surface-active properties of mixtures of N-tetradecyl-D-gluconamide sodium sesquisulfate with sodium sulfate, with carboxymethylcellulose, with sodium triphosphate, or with combinations of sodium sulfate and carboxymethylcellulose do

not vary markedly from those of the detergent itself. The addition of builders has, in some cases, also improved soil-removing ability. The implication is that the active compound may be materially extended by combination with a number of less expensive agents.

Experimentation is being continued on this group of compounds. If large-scale production of fatty gluconamide sodium sulfates for detergent use proves economically feasible, a considerable volume of both fatty acids and dextrose will be consumed. Additional outlets for two important agricultural commodities, fats and corn, would thus develop.

High-Flavored Sirup Introduced as New Maple Product

A process for giving maple sirup intensified maple flavor and color has been devised at the Eastern Regional Research Laboratory. It requires only heating under atmospheric pressure at elevated temperatures. Commercial maple sirup is boiled until enough water has been removed to raise the boiling point to between 240° and 250° F. The thickened sirup is held at this elevated temperature for 3 hours for "Fancy" grade sirup and a shorter time for the lower grades, but further loss of water during this holding period is prevented by using a tight lid fitted with a reflux condenser. When cooking is completed, as much water is added as was lost by evaporation. The sirup becomes darker and develops a maple flavor that is at least four times as strong as that of the original sirup. One part of this high-flavored sirup diluted with three parts of cane-sugar sirup makes a maple-sirup blend with a flavor that is hardly distinguishable from that of the original maple sirup. With the exception of a kettle lid provided with a long, upright pipe to serve as a reflux condenser, the process requires only the equipment available in an ordinary maple-sirup plant.

The process offers a means for preparing low-cost, full-flavored sirup blends of high quality. It should provide an expanded market for maple sirup and should solve the problem of utilizing the grades of maple sirup that are in surplus. The high-flavored maple sirup may provide a flavoring agent for uses in which ordinary maple sirup has not been successful because of the high proportion of sugar to flavoring material.

Permanent Color Standards Developed for Maple Sirup

To improve the grading of maple sirup, the Eastern Laboratory, in collaboration with the Sugar Branch of Production and Marketing Administration, has developed a simple color comparator fitted with permanent standards made of colored glass. The work was done under a Research and Marketing Act project.

Color has long been an important factor in grading maple sirup. The United States Department of Agriculture's color standards for maple sirup have been represented by solutions of caramel in glycerin, and the Department has supplied these standards each year for Federal and State inspection of maple sirup. Because the color of the caramel-glycerin solutions is not very stable, these color standards have not been entirely satisfactory.

The new comparator is a small metal box divided into five equal compartments, each of which has a square window at the front and at the back. The color standards are amber-color glasses that have been ground and polished so that their colors closely match the colors of freshly prepared caramel-glycerin solutions corresponding to the earlier maple-sirup standards. The glass color standards, designated "Light Amber," "Medium Amber," and "Dark Amber," are cemented to the front windows in the first, third, and fifth compartments. A 2-ounce bottle filled with a clear solution of glycerin in water is placed back of each glass standard. A similar bottle containing sirup to be graded is placed in either the second or fourth compartment, so that it is between adjacent standards. The comparator is held at a convenient distance from the eye and viewed in diffuse daylight; this permits the color classification of the sirup to be determined by comparing the sample with the standards.

To assist in grading cloudy sirups, three bottles containing suspensions of bentonite in glycerin and water are available. These correspond to different degrees of cloudiness permitted in the grades of maple sirup for table use.

The color comparator was made available by a commercial firm shortly before the beginning of the 1950 maple-sirup season; more than 200 were sold almost immediately.

Valuable Information Obtained in Studies of Sugar-Beet Juices

Although beet sugar can trace its history back to 1797 and has developed in the United States into a 300-million-dollar industry, knowledge of the composition of its raw material and of changes that occur during processing is incomplete. A composition study of diffusion juice, thick juice, and molasses from factories in Manteca, Calif., Rupert, Idaho, Sidney, Mont., and Centerfield, Utah, was undertaken by the Western Regional Research Laboratory under an RMA project to obtain quantitative information on composition changes which markedly influence the rate of crystallization of sucrose. The materials that cause molasses formation were of greatest interest, as they have a direct bearing on the amount of sugar that can be recovered from beets and thus on the financial return to the farmer. Also of interest was quantitative information on materials that could be recovered from the sediment resulting from the purification step and on materials that could be recovered from molasses. In getting this information, the influence of locality and harvesting time was also noted in a preliminary way.

To reach the goals set, it was necessary to have available techniques that permit determination of each constituent. Fortunately, many compounds could be determined by methods already published, but known methods for other compounds required so much time and effort that new ones had to be devised. A further obstacle was that hundreds of compounds had been reported to be present in sugar beets without indication of the amount or, in most cases, the precautions necessary to insure the absence of artificial substances.

The limit of concentration below which minor or trace constituents would be disregarded was set at 0.001 percent because the presence

of any constituent at concentrations below that point would be of little significance in the formation of molasses, and the constituent would have little or no economic value as a byproduct. With this limitation, there seems to be no more than 12 amino acids or their amides and 7 other organic compounds in sugar beets. Of these, only betaine, aspartic and glutamic acids and their amides, oxalic acid, citric acid, lactic acid, and malic acid are present in amounts greater than 0.005 percent in all the diffusion juices examined. Pyrrolidone carboxylic acid, which is derived from glutamine, is found in relatively large amounts in thick juice and molasses. Variation in composition during the harvesting season was slight. Juice from California beets contained the most impurities; that from Utah and Montana beets was next; and that from Idaho beets contained the least, or about half as much as the juice from California beets. California beets are harvested before they have reached a mature vegetative state, which might account for the difference.

As information on the kinds and quantities of substances in beets becomes available, new and old methods of purification can be examined for possible improvements.

A new, rapid method developed for the analysis of betaine is of interest to poultry producers. Several million pounds of betaine is available from waste liquors of the beet-sugar industry for possible use as a poultry-feed supplement.

Further work will be done on the composition of diffusion juices from beets representing the major producing areas of the country. Methods of increasing the purity of thick juice will be developed in the light of the knowledge obtained from this study.

Discovery of Crystalline Levulose Dihydrate Suggests New Method for Preparing Pure Levulose

During an investigation of the behavior of sugar solutions at low temperatures, in connection with the freezing preservation of fruits, a new crystalline hydrate of levulose (fructose) was recently discovered by the Western Regional Research Laboratory. This compound, which contains 2 molecules of water per molecule of levulose, was easier to crystallize and less soluble than anhydrous levulose. These properties suggested a method of preparing levulose which should have advantages over methods now used.

Levulose occurs in many fruit juices and in honey. It is sweeter than ordinary table sugar, and does not mask fruit flavors. These properties make levulose potentially useful in the food industry, but high cost has limited its use.

Levulose, now separated from invert sugar or the hydrolyzate of inulin, is first separated from dextrose and other impurities by precipitation as calcium levulose. After removal of calcium, the solution containing levulose (and some dextrose) is evaporated to about 90 percent solids, and anhydrous levulose is crystallized. Dextrose seriously inhibits levulose crystallization, reducing the yield and necessitating long crystallization periods (3 days for a two-thirds yield).

The proposed new method of preparing levulose starts with the solution obtained from the calcium-levulose compound as just de-

scribed. This solution is concentrated to about 65 percent solids, cooled to about 15° F., and seeded with crystals of levulose dihydrate. In a short time crystallization is complete, and the crystals are centrifuged and washed. Reconcentration and removal of another crop of crystals should be sufficient to leave the mother liquor saturated with dextrose.

The principal advantages of the proposed method are more rapid crystallization and higher yield, both being promoted by the absence of inhibition of crystallization by dextrose. Additional advantages include easier centrifugation and washing, and possible elimination of the need for decolorization with carbon. Crystallization is essentially complete in a short time, probably less than 2 hours with agitation. Two factors contribute to the high yield—nearly complete crystallization and low solubility of the dihydrate at 15° F. At this temperature the solubility (calculated for anhydrous levulose) is about half the solubility of anhydrous levulose at the crystallization temperature used in the present commercial method. As an example of possible recovery, 65 percent of the levulose originally present was obtained as a pure product by one crystallization from a solution containing 60 percent levulose and 6 percent dextrose. Concentration of the filtrate and a second crystallization would probably have increased the total yield to about 80 percent of the levulose originally present. More strikingly, the exhausted mother liquor from the present commercial process could be made to yield about half of its levulose content by one dihydrate crystallization at 15° F. Easier centrifugation and washing, as well as lower losses in washing, result from the lower concentration of the mother liquor. Preliminary results suggest that decolorization with carbon is not necessary with the dihydrate method.

Less evaporation is required to reach the optimum initial concentration, but this advantage may be offset by the necessity of removing water of crystallization to obtain anhydrous levulose. The low melting point of levulose dihydrate would prevent marketing it as a dry powder, but for many purposes it could be sold as an 80-percent "liquid sugar" without further drying.

The suggested method is based on solubility data and preliminary laboratory experiments. The disadvantage of working at low temperatures would seem to be outweighed by the higher yield and more rapid crystallization; however, pilot-plant work and adequate production, cost, and marketing studies will be required to evaluate the commercial feasibility of the method.

HONEY PRODUCTS

New Honey Products Developed

Although honeybees are frequently kept primarily for their service in pollinating orchard trees, market outlets for their honey add to their usefulness. Providing as many market outlets as possible, so that the bee population will be maintained at a high level, is the objective of the honey projects under the Research and Marketing Act at the Eastern Laboratory.

In cooperation with the Bureau of Dairy Industry, the Laboratory has developed a dried honey-skim milk product. Because honey con-

tains a high percentage of the hygroscopic sugar levulose, it is not feasible to incorporate more than 50 percent of honey solids in the final product, and 40 percent is better. Honey and dried skim milk can be mixed to a granular product, which can be tunnel-dried and ground. Or a similar mixture, thinned with water, can be spray-dried. Part of the water in the honey combines with some of the sugars to form crystals of lactose and dextrose hydrates. The honey-milk product should find use in the confectionary and baking industries.

Another new product is a honey-fruit spread. To prepare it, concentrated fruit juice or puree is mixed with honey, concentrated to 80 to 85 percent solids, and then stored for a few days at such a temperature that crystals of dextrose hydrate will form. The product is a smooth, fine-grained spread, in which the fruit flavor predominates. In fact, as honey is principally a source of sweetness and of the dextrose crystals that make the product a spread, the honey flavor is really not desirable. Hence the honey used is preferably one of mild flavor or one that has been largely deflavored.

This provides an outlet for another honey product. A good deal of the honey produced has such a strong flavor that it finds few or no buyers. Previously known processes for deflavoring honey with bentonite or with activated carbon have been improved to such an extent that it is now feasible to rework such honey into a partially or wholly deflavored product. This modified honey is well suited for making the honey-fruit spread.

ALFALFA

Means Sought for Preserving Nutritive Values in Alfalfa

Preservation of alfalfa's high nutritive value during processing and storage is of great importance to the livestock and poultry industries. A good grade of alfalfa is not only an important source of protein, but it is particularly rich in a wide variety of dietary factors, normally supplied by green forage. Because it is the most practical source of beta-carotene (precursor of vitamin A) for animal and poultry feeds, the preservation of this constituent is one of the most pressing problems of the alfalfa industry. The Western Laboratory is trying to solve it.

Most alfalfa is field-cured, and the loss of carotene is tremendous—often 50 or 75 percent during harvesting alone. A small fraction of the crop is artificially dried with little loss of carotene. A well-controlled drier can dehydrate alfalfa with a loss of not more than 5 to 15 percent of its carotene content. Storage of dehydrated alfalfa meal for 6 months at normal temperatures results in a loss of two-thirds to three-fourths of its carotene content through oxidation.

There are three obvious approaches to the problem of reducing these losses: (1) Storing in an inert atmosphere, (2) storing at reduced temperatures, and (3) adding chemical antioxidants. The first two methods have been used commercially on a limited scale. They have the disadvantages of high cost of installation and lack of carotene protection between the time the meal is removed from storage and the time it is consumed by the animal. The third method, that of treating

alfalfa with chemical antioxidants, does not present the disadvantages of the other two. Development of such a method requires (1) screening a large number of compounds for antioxidant activity, (2) determining the possible toxic effects of selected ones on animals, and (3) devising means for applying the compounds in the field. Seventy-five antioxidants which have found use in the petroleum, rubber, and food industries have been used on alfalfa, and a few have shown considerable promise. These are 2,5-ditertiary butyl hydroquinone, the corresponding amyl and benzyl derivatives, 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline, and the corresponding 6-phenyl derivative. Certain of these are being tested for their toxicity toward rats and chicks, and a study of their field application is in progress.

The beta-carotene of alfalfa exists as 3 principal stereoisomers—all-trans, neo-B, and neo-U—having relative vitamin-A potencies of 100, 53, and 38, respectively. An excellent method has been devised for analyzing alfalfa products for these isomers, and the application of the method has shown a number of interesting relationships. Most of the carotene in freshly cut alfalfa is in the most nutritious form, 85 to 90 percent being the all-trans isomer. The carotene-isomer equilibrium does not change greatly with sun curing, although total carotene is decreased markedly. Artificial dehydration, because of heating, isomerizes the carotene to give 45 to 55 percent all-trans, 30 to 40 percent neo-B, and 10 to 15 percent neo-U, with a resultant 15 to 20 percent loss of nutritive value for this vitamin. Irradiation of alfalfa meal changes much of the neo-B back to the all-trans form. This effect may have some commercial application.

Research by collaborating agricultural experiment stations shows that including alfalfa in the diets of swine yields growth responses in excess of those expected from the known nutrients of the plant. The Western Laboratory initiated research to identify these unknown growth factors. A combination of microbiological and chromatographic assay techniques showed that dehydrated alfalfa contains 4 to 6 parts per billion of vitamin B₁₂. Although poultry and swine require 5 to 10 parts per billion of this vitamin, their rations normally contain but 5 to 20 percent of alfalfa, which would satisfy only a portion of their vitamin-B₁₂ requirement. Preliminary data show the presence of two additional unidentified factors in alfalfa which may have nutritional significance.

CHEMICAL PRODUCTS FROM TOBACCO

Tobacco Research Speeded by New Techniques

Recent research at the Eastern Laboratory shows that, in addition to nicotine, cigar-leaf tobacco contains several alkaloids in small quantities. Their detection was made possible by the newly developed technique of paper chromatography. In model experiments, in which known compounds were used, nicotine, nornicotine, and 11 of their transformation products were separated. Application of the technique to the alkaloids extracted from unfermented cigar-leaf tobacco confirmed the presence of nicotine and nornicotine, and revealed appreciable quantities of three other alkaloids not yet chemically identified. Investigation of alkaloids from fermented tobacco showed a

qualitative similarity to the mixtures obtained by oxidation of nicotine, either with hydrogen peroxide or photochemically in the presence of methylene blue. This study may elucidate the mechanism of nicotine degradation during the fermentation of tobacco.

In research on the chemical composition of tobacco, the aromatic fraction from flue-cured tobacco has been extracted and studied. Chromatographic examination of some of the fractions indicated the presence of lactic acid, a constituent hitherto unreported.

The tobacco alkaloids and related compounds have been characterized recently by ultraviolet-absorption spectroscopy. Ultraviolet-absorption data have proved of great value, in conjunction with the chromatographic technique, for identifying alkaloids, for elucidating the structure of "N-methyl myosmine," and as the basis of a new spectrophotometric method for determining nicotine in tobacco products. This new optical method is far more rapid and more sensitive than previously used chemical methods. As a result, research on the composition of tobacco and on recovery of nicotine from *Nicotiana rustica* has been more efficient.

New Process Recovers Nicotine From Green *Nicotiana rustica* Plants

As pointed out in the 1948 report, the Eastern Laboratory has been studying, under a Research and Marketing Act project, the recovery of nicotine from *Nicotiana rustica*. This plant contains two or three times the amount of nicotine found in ordinary tobacco. As a source of nicotine it can be six or more times richer than the tobacco stems currently used.

It has been shown that nicotine could be recovered from green *rustica* by a distillation process similar to that in commercial use for tobacco stems. As *Nicotiana rustica* would be available in a green state, whereas tobacco stems are only available dry, it was thought that a simpler process might be developed for the former material. Such a process has now been developed and evaluated in an integrated pilot plant.

The plants, harvested just above ground level, are fed—stalk and all—into a fodder cutter. The juice is expressed from the chopped material by passing it repeatedly through a cane mill in about the same way that juice is expressed from sugarcane. A continuous rotary press may be substituted for the cane mill if the plants are first broken up in a hammer mill. The expressed juice will contain about 92 percent of the nicotine that was in the plants. To free the nicotine and to permit storage of the juice throughout the year, 1.5 percent of lime is added. To recover the nicotine, the supernatant juice is separated from the sediment, the sediment is washed with water to remove remaining juice, sodium carbonate is added to the combined juice to complete the precipitation of colloidal matter, and the juice is filtered hot. The nicotine can then be extracted from this juice as 40-percent nicotine sulfate by the procedure now used for making nicotine sulfate from distilled tobacco stems. This procedure entails contacting the juice in a packed tower countercurrently with kerosene and then extracting the nicotine from the kerosene with sulfuric acid.

The new process does away with the necessity for drying the raw material. The entire green crop must be converted to juice during the harvesting season, but the limed juice can be stored throughout the year without significant loss of nicotine. This makes it possible to carry out the succeeding steps throughout the year.

The new process for extracting nicotine makes it possible for *Nicotiana rustica* to become a practical source of nicotine, if its culture in this country can be made economically feasible.

Tobacco-Flavor Concentrates Produced

The substance responsible for the fragrance of tobacco stems has been recovered in a concentrated and usable form by a process developed at the Eastern Regional Laboratory. Because tobacco is stored in tight hogsheads for some time before the leaves and stems are separated, the stems have about the same fragrance as the leaves.

After preliminary experiments with various extraction solvents (water, acetone, chloroform, benzene, and tertiary butyl, methyl, isopropyl, and ethyl alcohols), ethyl alcohol was chosen as the best. In preparing the concentrates, the stems are submerged in the nearly boiling alcohol for several hours. After the extract is separated from the stems, the alcohol is removed by distillation, leaving a solution that contains not only the fragrant substance but also sugars, salts, nicotine, and coloring matter. Some of the salts crystallize and can be filtered off. The nicotine—89 percent of that in the tobacco—can be removed if desired. The final solution—tobacco essence—is then ready for use.

When sprayed on paper or wood, the essence imparts the fragrance of tobacco which is retained for some hours or days, depending on the degree of exposure to air. The nature of the fragrance reflects the kind of tobacco used—Cuban, Connecticut Broadleaf, and so on. Essence from leaves is somewhat better than that from stems, but not markedly so.

Stems from 10 varieties of cigar tobacco and 3 varieties of cigarette-type tobacco were used for the preparation of extracts, which were evaluated as to intensity and quality of fragrance. The varieties were rated in the following descending order: Connecticut Broadleaf, Connecticut Shade, Red Burley, Imported Cuban, Imported Cuban Wrapper, Pennsylvania Filler, Flue Cured, Havana Seed Leaf, Light Burley, Wisconsin, Sumatra, Ohio Broadleaf, and One-Sucker.

The new product is attracting attention from tobacco firms, not only for imparting tobacco fragrance to paper or wood containers, but also for boosting the aroma of some tobacco products.

MISCELLANEOUS VEGETABLE PRODUCTS

New Information Helps Explain Softening of Cucumber-Pickle Stock

For many years an explanation has been sought for the softening of cucumbers held in salt brine for pickling, which is responsible for the loss of a quarter-million dollars annually to pickle manufacturers in the southeastern brining area alone. As reported in 1949, the cause of this lack of firmness in fermenting cucumbers has been established as

enzymatic in nature, and a test for early detection of the softening enzyme has been developed.

These were important steps toward a solution of the problem, but much additional information was needed to completely eliminate the spoilage caused by softening. Some of this information has been acquired by scientists at the Bureau's Food Fermentation Laboratory in Raleigh, N. C., and Enzyme Research Division in Albany, Calif. These two laboratories are collaborating in a thorough study of fruit-softening, or pectin-splitting, enzymes in an effort to measure the activity and find the source of the one responsible for the softening of cucumber-pickle stock.

Last year samples of brine and softened cucumber stock were collected, and studies of softening were made in many of the pickling plants throughout the southeastern area. Brines were also sampled in northern pickling plants, although softening was believed to be less serious in that section because lower temperatures prevail during the period when cucumber-pickle stock is cured and stored.

In all, 300 vat brines from 21 pickling stations located in 9 major pickle-producing States were examined. From the results, it was evident that softening may occur over a widespread geographical area and is not restricted to any one particular area where cucumbers are brined.

Micro-organisms responsible for fermentation of the cucumber have been considered the most likely source of the softening enzyme in brines. So far, two groups of yeasts associated with cucumber fermentations have been thoroughly investigated. The studies showed, however, that neither the yeasts that cause film formation on brines nor those that cause gaseous fermentation during brining are the source of the objectionable enzyme.

It was found, further, that the cucumber itself may play an important role in the softening problem. Pectin-splitting enzymes were found in green cucumbers and in various parts of the cucumber plant. A softening enzyme was detected in large amounts in the dry seeds of several varieties of cucumbers, and in both pollen and pollinated cucumber flowers. The last source mentioned is especially significant because at times, and with certain varieties more than others, the flowers remain attached to the smaller cucumbers when they are brined. The softening enzyme also was found in ripening cucumbers. If liberated into the brine, this enzyme could seriously influence the firmness of the stock.

This new knowledge of the biochemical changes that occur in cucumber brining provides a basis for additional research directed at controlling the enzyme and improving the brining process to eliminate the costly softening of cucumber-pickle stock.

Alfalfa Driers Useful for Drying Vegetable Wastes

A process for preparing high-grade, vegetable-leaf meals from field wastes was published by the Bureau several years ago. In this process, through-circulation, apron driers were used. The process did not find wide industrial use, principally because moderate temperature, apron driers are inherently costly and are not as widely used in farm operations as are rotary driers. Direct-heat, rotary driers, as used

for alfalfa, are cheaper per unit of evaporating capacity, are simple to operate, and are widely used for processing farm crops. The Eastern Regional Laboratory has therefore investigated the suitability of this type of drier for preparing high-carotene, high-protein leaf meals from vegetable wastes. A small commercial-scale, portable, alfalfa drier having an evaporating capacity of 2,500 pounds of water per hour was used. Auxiliary equipment consisted of a fodder cutter having lawn-mower-type blades for chopping the fresh waste and a vibrating screen for separating the dried leaf meal from the partially dried stems.

Pea vines, lima-bean vines, and broccoli, spinach, and beet-top wastes have been processed in this unit at several inlet-gas temperatures to determine the conditions for maximum yield of high-quality product. On the basis of carotene retained and carotene recovered in leaf meal per ton of waste, high-temperature rotary drying compares favorably with, and at times excels, through-circulation drying at moderate temperatures in apron driers. The various leaf meals produced under the recommended drying conditions contained the following principal feed values: Broccoli, 518 p. p. m. (parts per million) carotene and 40 percent protein; spinach, 361 p. p. m. carotene and 32 percent protein; beet tops, 264 p. p. m. carotene and 21 percent protein; lima bean vines, 203 p. p. m. carotene and 15 percent protein; pea vines, 115 p. p. m. carotene and 17 percent protein. The carotene figures are probably conservative owing to a delay of about 5 to 6 hours between the time of harvesting and when the wastes were processed. This is especially true of the pea vines and lima bean vines because of their bruised condition when discharged from the viners. A typical, good grade, alfalfa meal would contain about 135 p. p. m. carotene and 17 percent protein.

Since the raw vegetable wastes that were tested varied widely in physical form, it is probable that field wastes from other vegetables can also be successfully processed to leaf meal in this way. Costs per ton of finished meal, for this type of drier, can reasonably be expected to be lower than for an apron-type drier. The rotary type, alfalfa drier can also be used for drying potatoes to make feed and certain types of flour and for drying buckwheat plants to prepare leaf meal as a source of rutin. When the drier is used for these different purposes, the cost of producing each of the products is reduced, since the fixed charges can be spread over the entire year.

Refinements Made in Sweetpotato-Starch and Feed-Protein Process

Further refinements were made in an improved and simplified process for the extraction and purification of sweetpotato starch at the Southern Regional Research Laboratory during the year. These refinements have increased the economy and the efficiency of the process for commercial exploitation when the costs of growing sweetpotatoes for industrial use are sufficiently reduced, or when an adequate supply of offgrade sweetpotatoes is available from fresh-vegetable-market rejects.

Use of a saw-blade rasp as the first grinder, followed by a screen to remove coarse pulp fragments of low-starch content, reduces the cost and power requirement at this stage of the process and appears to

make easier the elimination of fine pulp or fiber in the finished starch. Use of an improved continuous centrifugal to separate most of the soluble matter and some of the low-starch material from the rasped pulp before the second grinding and screening appears to increase the capacity of the plant and to further simplify the starch-refining process. Improved procedures for chemical control and for determining the insoluble impurities accompanying the starch have simplified the production of a uniformly high-quality product at minimum cost.

Five runs in the pilot plant at the Southern Regional Laboratory were made during the year, in which particular attention was given to the recovery of protein as a byproduct of starch manufacture. A sufficient quantity was produced to prepare about 750 pounds of protein-enriched sweetpotato pulp to complete additional calf-feeding experiments undertaken by the Florida Agricultural Experiment Station during the year. About 900 pounds of plain sweetpotato pulp also was prepared for use in these experiments. The animals ate the feeds well and, at the last report, were thriving.

Besides smooth operation of the pilot plant and the production of higher-quality products, the year's investigations provided fundamental data on the chemical and physical properties of sweetpotato starch which better explain its advantages over competitive products in certain specialized uses, such as storage batteries. These data also were useful in exploring the possibilities of additional uses for which the starch may be suitable. A relatively crude sweetpotato starch, for example, appears promising for use in oil-well drilling.

FRUITS AND FRUIT PRODUCTS

Frozen Apple-Juice Concentrate Prepared by Three Methods

Development work on the production of frozen apple-juice concentrate is being carried out at the Western Regional Research Laboratory. The project, financed by Research and Marketing Act funds, involves experiments with various methods of producing a concentrate of natural and desirable flavor, determination of the keeping quality of the product in frozen storage, and development of an economical process.

Frozen apple concentrates have been prepared by three different methods, each of which appears to be technically suitable for commercial use. These methods are as follows: (1) Vacuum concentration to sixfold followed by dilution with fresh juice to produce a fourfold concentrate. This is similar to the method currently in use for commercial preparation of frozen orange concentrate. (2) Removal and concentration of the volatile flavor constituents (essence), vacuum concentration of the stripped residue to slightly over fourfold, and final addition of the essence to the concentrate. (3) Preparation of essence as in (2), concentration of stripped residue to sixfold, and addition of essence and fresh juice to produce a fourfold concentrate. Methods (2) and (3) employ the Eastern Regional Laboratory's essence-recovery process, which involves vaporization of about 10 percent of the juice and concentration of the vapors to at least a hundredfold, based on volume of original juice.

Concentrates prepared by methods (2) and (3) were of excellent quality, being superior in flavor and aroma to those prepared by method (1). No significant differences in either flavor or aroma were found between samples prepared by method (2) and those prepared by method (3).

It was found that the stripped juice could be concentrated to the desired degree at temperatures as high as 130° F. without impairment of flavor, the judgment being based on the organoleptic quality of the juice after being reconstituted with water and essence. When concentration was effected at 145° F., however, a distinguishable off-flavor was developed. The results indicate that the concentration can be carried out in relatively low-cost vacuum pans without affecting the flavor of the product.

Storage studies on concentrates prepared by the three methods are in progress. After 5 months' storage at 0° F., samples prepared by methods (2) and (3) were again found equal to each other and superior to samples of frozen apple concentrate obtained through use of method (1). The organoleptic tests showed no evidence of loss in original flavor of any of these samples during this storage period.

On the basis of available data, methods (2) and (3) appear equally suitable for commercial use. Unless significant differences in the keeping quality of the products become apparent at later stages of the storage study, the choice between two methods will depend largely on economic considerations.

New Equipment Developed for Flash-Heating of Juices

A new direct steam-injection heater has been developed by the Western Regional Laboratory for use in the recovery of volatile essences from heat-sensitive fruit and vegetable juices and purees. It has shown great promise in applications where rapid heating and cooling are essential. In combination with vacuum evaporative cooling, the system becomes an unusual device for high-temperature, short-time heat processing, which includes pasteurization, sterilization, and inactivation of enzymes. Heating is done under conditions not practically attainable with previously available equipment. Numerous runs have been made where the fluid has been heated from room or storage temperatures to as high as 300° F. and then cooled to room temperature within a total elapsed time of 0.6 second. Many materials that are ordinarily damaged by heating may be heated in the new system without detectable impairment of flavor. Since no heat-transfer surfaces are involved, fouling is not a problem. Purees as well as juices can be handled in the system without burning of materials on hot surfaces. Vapors evolved during vacuum evaporative cooling may be concentrated and returned to the product if the volatile components are desirable flavor constituents, or the vapors may be discarded if the volatile components are undesirable. Experimental tests indicate that most micro-organisms of the type that ordinarily causes spoilage in fruit products are destroyed at retention times of less than 1 second at temperatures in the range of 220° to 250° F. Oxidative enzymes appear to be inactivated by the same treatment.

For products that are not extremely heat sensitive, the steam-injection heater may be used as a component of an atmospheric-pressure, heat-processing system which is a substantial improvement over conventional tubular or plate types of heaters and conventional types of evaporators or concentrators. The improvement is most significant in the processing of materials that foul ordinary heaters at an objectionable rate or to an objectionable extent. The improved system consists of a steam-injection heater coupled directly to a tubular heater. Material leaves the injection heater as a mixture of vapor and liquid and passes through the tubular heater at high velocity, thus minimizing fouling, which ordinarily occurs in the low-velocity sections of heaters. The high-velocity effects a scrubbing action to keep heated surfaces clean and contributes to high heat-transfer rates. Extremely difficult materials, such as pea puree, have been handled successfully in the system for the recovery of volatile flavor components; no deposition of materials on the heated surfaces occurred, even during operations in which high heat-transfer rates were maintained. The combination described has interesting possibilities as a short-time, single-pass concentrator for heat-sensitive purees and other materials, with or without a vacuum cooler for the concentrate.

Commercial Production of Fruit Essences Expanding

With the passage of a Federal law on August 17, 1949, to permit the manufacture, under specified conditions, of fruit essences "without the payment of the \$9 per gallon tax" formerly imposed, the commercial adoption of the Eastern Regional Laboratory's process for the recovery of volatile flavoring constituents, or essence, from fruit juices has greatly expanded.

About 50 companies have installed essence-recovery units. One company has three units, and another company reports it can treat 5,000,000 gallons of apple juice annually for the recovery of the essence.

The process has been adapted by industry to recover the essence from grapes, cherries, peaches, blackberries, strawberries, pineapples, and apricots. All of these essences have been made commercially. In the cases of apples and grapes the essence is being sold either in combination with the concentrated juice or separately. In addition, industry has produced on an experimental scale essences from oranges, tomatoes, maple sirup, raspberries, Damson plums, and quinces. Preserve manufacturers have recovered essences from various fruits. Apple processors have reported promising results on the recovery of essence from apple peels and cores.

The Eastern Laboratory has applied the process, originally developed for apple juice, experimentally to the juices of other fruits such as grapes, strawberries, blackberries, raspberries, huckleberries, and peaches. It has now perfected the process for Concord grapes.

The essences are now being used commercially in the preparation of carbonated and noncarbonated beverages, flavoring extracts, and candy, and in frozen, concentrated grape juice. A number of manufacturers of both frozen concentrated apple juice and grape juice are evaluating the application of fruit essences to ice-cream products,

ice-cream toppings, table sirup, soda-fountain sirups, pharmaceuticals, and fruit-flavored sirup bases for subsequent reconstitution and distribution by dairy companies on their milk routes.

Sanitation Survey Made of Citrus Processing in Florida

Numerous Florida citrus-fruit canneries requested that bacteriologists of the Agricultural Chemical Research Division survey sanitation conditions in their preparation and canning lines with a view to producing products of the highest quality that is possible. They wanted to know what yeasts and molds might be encountered at each stage of juice production, at what points they are most likely to enter, and at what rate and under what conditions they multiply. They also wanted to know the full effect of such micro-organisms on flavor and quality. While many organisms do not threaten health, their presence nearly always impairs flavor.

In a survey conducted in response to these requests, examinations were made of the incoming fruit, the washed fruit, the juice extractors, the screens—in short, scrutiny was made of all possible sources of microbiological contamination. In general, plant sanitation was considered good. One practical discovery was that the side cover on a juice extractor interfered with completely effective cleaning and was a gathering and growing place for yeasts, molds, and bacteria.

It was also found that juice troughs had dead ends where juice and pulp accumulated because they were not drained during the period of operation, such places furnishing micro-organisms with ample food and opportunity for growth. Improvements were effected by eliminating dead ends in juice troughs, redesigning extractors so they could be thoroughly cleaned, and shortening juice lines so that the least possible time is needed to convey juice from the extractors to holding tanks. Now many operators are holding the juice in cold-walled tanks when necessary. More attention is also being directed to the sanitation of condensing equipment producing frozen-juice concentrates. It is possible that organisms growing in the more inaccessible places can contribute to the off-flavor found intermittently in the finished product.

The application of results of the survey will perhaps be of the greatest value in plants producing frozen orange juice concentrates, where pasteurization is not used, and consequently it is essential that counts of micro-organisms be kept below the permissible value in order to have products of high quality and to meet public-health requirements.

Separation of Sugar Prevented in Frozen-Fruit Spreads

Recent research at the Western Regional Research Laboratory has disclosed the cause of, and a remedy for, a phenomenon which has hindered commercial acceptance of the cold-processed, frozen-fruit spreads first described in the reports for 1946 and 1947. These jelly-like spreads are made from fresh fruits by a process that requires no heating. They retain the natural flavor, color, and aroma of the fresh fruit.

The difficulty has been that during freezing storage unattractive, white, moldlike spots form and grow. At the same time the desirable texture of the spread is gradually lost.

The moldlike growths, which also sometimes occur in ordinary frozen fruits, were found to consist of crystalline clumps of a sucrose hydrate, stable only at low temperatures and previously unknown. Although these sugar crystals are, of course, harmless, their appearance is sufficient to spoil the marketability of the frozen-fruit spread.

Substitution of other sweetening agents for part of the sucrose was tried as a means of preventing or retarding the formation of sucrose hydrate. Experiments were made on sugar sirups alone, in which part of the sucrose was replaced with other sweetening agents, and similar experiments were made with fresh-fruit spreads, in which part of the sucrose normally used in making the spread was replaced with levulose. The sirups and spreads were subjected to various refrigeration temperatures. Summarizing the results, it may be stated that if 30 percent of the normal sucrose requirement for the fruit spread is replaced by invert sugar, and if the product is prepared and packaged under conditions that will prevent evaporation and seeding with sucrose-hydrate dust (minute airborne crystals), no growth of crystals will occur during a year's storage at 0° or -10° F.

Sucrose-hydrate growth in sirup held at -10° F. was found to be reduced (even though the sirup was seeded) when part of the total sugar was replaced by corn sirup, levulose, maltose, or invert sugar. Dextrose was unsatisfactory, because its monohydrate frequently crystallized spontaneously from the cold sirup. In sirups containing 60 percent or less of total sugars, replacement of 30 percent of the sucrose by corn sirup or maltose appeared to be satisfactory, whereas, at a total sugar concentration of 70 percent, replacement of 40 percent or more of the sucrose was required. In the sirups, levulose and invert sugar were less effective than corn sirup and maltose in checking sucrose-hydrate growth.

When the frozen-fruit spreads were tested, it was found that sucrose-hydrate formation can be eliminated by proper selection of sugars, use of an air-tight package, and precautions against accidental seeding. Unseeded samples packed in hermetically sealed containers did not develop the crystals, but unseeded samples packed in paper cups did develop them, perhaps because of gradual loss of moisture through the container walls. Even in the paper cups, however, replacement of 30 percent of the sucrose with invert sugar was effective during a year's storage at -30°, 0°, and 10° F. Trace amounts of crystals appeared in the samples stored at -10° F. When the amount of invert sugar was raised to 50 percent, crystals of glucose hydrate formed at -10°. The combination of hermetically sealed containers, avoidance of seeding, and 30-percent replacement of sucrose by invert sugar, therefore, gives good assurance of stability in the normal range of storage temperatures for frozen foods, -10° to 0°.

The investigation will be extended to include tests of corn sirups as inhibitors of sucrose hydrate formation in frozen-fruit spreads.

Frozen Concentrates Made From Texas Grapefruit

Pioneer work undertaken at the Bureau's Fruit and Vegetable Products Laboratory in Weslaco, Tex., during the past year has already been of considerable help to grapefruit growers and processors interested in the development of a frozen citrus concentrate industry in the Rio Grande Valley.

This work was undertaken after the phenomenal success of frozen orange concentrate had established this type of product in the food market. Although the production of oranges in Texas is too limited to maintain processing plants, grapefruit (produced in quantities five times as great) has for some time supported a sizable industry for canning unconcentrated grapefruit juice.

Methods by which high-quality concentrates are prepared from oranges do not produce an acceptable grapefruit concentrate in any area. In Texas, the production of grapefruit concentrate is further complicated by the fact that a large quantity of the grapefruit grown is pink- or red-fleshed, rather than white. Since these varieties command a premium price on the fresh-fruit market, growers have increased their plantings steadily. In recent years, they have begun to look to processing as an outlet for that portion of the crop not absorbed by the fresh-fruit market. But the canning of juice from pink and red fruit has not been successful, because the juice has a muddy, unattractive appearance. Consequently, pink or red grapefruit that is not taken by the fresh-fruit market has become an economic problem.

Frozen grapefruit concentrate could not be expected to relieve the situation much unless the reconstituted juice had an attractive appearance. Therefore this was one of the first problems attacked by the Bureau's staff at Weslaco. After a thorough study, the scientists found that a distinctive pink color, suggestive of the fruit itself, can be obtained by incorporating a small amount of the grapefruit pulp in the concentrate. Concentrates produced experimentally at the Weslaco Laboratory also preserved the mild flavor generally considered to be one of the most delightful characteristics of pink or red grapefruit. With these qualities, concentrates from pink or red grapefruit should command a premium on the concentrate market comparable to that commanded by the fresh fruit in the fresh-fruit market.

While frozen grapefruit concentrate is too new in Texas for an accurate appraisal of its value to growers and processors, leaders in the citrus industry expect it to be an important factor in maintaining a stable market for the fruit. Studies are being continued at the Weslaco Laboratory in an effort to give commercial plants scientific guidance in the development of a commercial concentrate of the highest possible quality.

Sulfur Compounds in Orange Juice Identified

The nature of the sulfhydryl compounds in citrus juice was studied by the Enzyme Research Division in consideration of the fact that such substances frequently modify the odors and flavors of foods in which they occur; furthermore, they are often easily susceptible to

chemical changes whereby disagreeable odors and flavors may be produced.

The identification of the sulfhydryl compounds of orange juice as the amino acid, cysteine, and the tripeptide, glutathione, has been completed. The method used for the isolation and identification was briefly as follows:

The sulfhydryl compounds of fresh, clarified orange juice were precipitated as mercury salts, followed by regeneration of the sulfhydryl compounds. A repetition of this procedure gave a product of higher purity. The final preparation was then dried in the frozen state. Upon reacting this dry powder in liquid ammonia with sodium, followed by benzyl chloride, two crystalline sulphur-benzyl compounds were obtained which were separated by differences in solubility. These compounds were identified by their crystalline form and elementary analyses as derivatives of cysteine and glutathione.

Since the isolation of these derivatives was not quantitative, it was important to ascertain if all of the sulfhydryl in the regenerated mercury precipitates was present as cysteine and glutathione. The colorimetric method of Sullivan, which is specific for free cysteine, was used for this purpose. Since glutathione is composed in part of cysteine, the use of this method before and after hydrolysis measured both the cysteine and the glutathione. After devising methods to avoid the decomposition of sulfur compounds during the hydrolysis, it was found that essentially all of the sulfhydryl sulfur precipitated by mercury was either cysteine or glutathione. Since the mercury reagent precipitated approximately 80 percent of all such compounds in the orange juice, it is probable that the two substances now identified are the only sulfhydryl compounds in the juice.

The relative amounts of these two compounds was determined in more than a dozen lots of juice from both navel and Valencia oranges. It was found that the weight ratio of glutathione to cysteine was not 12 : 1, as previously reported, but varied from 2 : 1 to 1 : 2. No significance has yet been attached to this variation.

The identification of these compounds will now permit an investigation to determine whether or not they are the precursors of the hydrogen sulfide known to occur in fresh and processed orange juice, the ultimate goal of which is to prevent the formation of this and other sulfur compounds having bad odors.

Improved Products Made From Navel Oranges

Navel oranges constitute more than one-third of the total California orange production, and up to the present time they have not been suitable for processing, because the juice has a tendency to become extremely bitter shortly after being processed. When navel oranges are reamed or crushed, a water-soluble precursor of limonin is extracted from the albedo, section membranes, and fibrovascular bundles of the fruit. The chemical structure of the precursor remains unknown, but, on standing or being heated in the acid juice, this precursor changes into the insoluble dilactone limonin, which is believed to impart the characteristic bitter taste to the navel-orange products.

Studies made at the Fruit and Vegetable Chemistry Laboratory, Pasadena, Calif., during recent seasons have suggested two possible

methods of destroying or removing the bitter substance in navel orange juice, concentrates, and purees. One method removes the precursor of the bitter principle, or both the bitter principle and its precursor, by means of adsorption, while the other makes use of enzyme preparations to convert the bitter principle into unoffending compounds.

From present knowledge of the structure of limonin and its solubility properties, it would be expected that adsorbents such as activated charcoal would remove this substance from the juice. Experiments indicated that some activated carbons were effective in adsorbing not only the bitter compound, but also its precursor. Only relatively soft carbons of high adsorptive activity were effective. The characteristic orange flavor of the juices was not greatly impaired by the charcoal treatment.

The second approach to debittering navel-orange products involved treatment with pectic-enzyme preparations. Two types of pectolytic enzymes were employed. One was a commercial fungal enzyme preparation commonly used in the clarification of fruit juices, while the other was an enzyme complex prepared from the tomato fruit. Studies of enzymatic debittering have met with difficulties due to variations in the bitterness of navel oranges from season to season and the unreliability of taste tests. However certain facts appear to have been tentatively established. Both the fungal and tomato enzyme preparations will, under certain conditions, debitter navel-orange products, although the fungal enzyme preparation tested had the disadvantage of causing the development of off-flavors in the treated products. The length of time required for debittering by these two enzyme preparations varied from several hours to several days in the temperature range of 38° to 50° F. At a temperature of -10° F., the time required may be several months. The debittering action appears to be most effective in products that are most bitter and relatively high in pulp contents, such as orange purees. It appears to be the least effective in screened, unconcentrated orange juices.

The enzymatic debittering of navel-orange products is nearly always associated with the phenomenon of clarification or loss of "cloud." Since no direct action of the enzymes on limonin can be detected, it seems most likely that the mechanism of debittering involves a change in the colloidal state of the system. Pectic substances are good stabilizers of colloids, and their enzymatic breakdown may result in a shift of limonin from the colloidal phase to the insoluble-solid phase, accompanied by loss of the bitter taste. This belief is supported by the observation that unpasteurized navel-orange products occasionally undergo self-debittering over long periods of storage at about 38° F. Also, this phenomenon is associated with loss of cloud as a result of the action of the natural pectin-splitting enzyme (pectin-methylesterase) of the orange.

Further work of both fundamental and practical nature must be carried out before the possibility of applying either of these two methods for debittering navel-orange products on a commercial scale can be evaluated fully.

Domestic Deglet Noor Dates Canned and Pasteurized

Although dates have been used as food since prehistoric times, no wholly satisfactory method for their packaging and preservation had been developed until this year. Most dates sold at retail in this country are marketed in small cellophane-wrapped cartons or in cellophane bags. Dates marketed in this manner are usually dried to about 25 percent moisture content to prevent spoilage during storage, and since air can penetrate the cartons, the dates tend to darken slowly due to oxidation. Undried dates or hydrated dates containing 30 to 35 percent moisture are softer in texture and are preferred by many people over the drier dates. In order to make these softer dates available on the market in such a form that they will not spoil or turn dark, the Fruit and Vegetable Chemistry Laboratory at Pasadena, Calif., undertook to develop an improved method for preserving them in suitable containers.

An obvious approach to this end was the pasteurization of dates in hermetically sealed containers such as tin cans or glass jars. Therefore, research has been underway to learn the causes of spoilage and darkening of dates, to develop a pasteurization process that would prevent this spoilage, and to develop a packaging method that would prevent changes in color, texture, and flavor.

Darkening ("browning") of dates has been commonly attributed to the caramelization of sugars, or to the Maillard type of reaction which involves reducing sugars and amino acids. In order to determine the actual causes of the browning, dates were packed in hermetically sealed cans in air and under vacuum, pasteurized in boiling water, stored at 85° F., and examined after various lengths of time. From these experiments, it was concluded that darkening of pasteurized dates is primarily an oxidative reaction, perhaps involving the date tannins located just under the skin, rather than a caramelization or Maillard reaction. The results also indicated that dates can be pasteurized without seriously affecting their color if oxygen is excluded from the package by some method such as sealing under a vacuum.

Although dates pasteurized in a vacuumized container were found to retain a desirable appearance with regard to color, it was found that the use of a vacuum had a deleterious effect on the texture of the dates. Dates of 30 percent moisture content pasteurized under a vacuum appeared to be bloated, and the skins were badly split and loosened. The flesh appeared to be gassy, although odor and flavor were normal. This effect is peculiar to the higher-moisture dates and is practically absent in dates of 25 percent or lower moisture content similarly pasteurized under a vacuum.

To retain the advantages of eliminating oxygen from the container and to avoid the rupturing effect of pasteurizing dates in a vacuum, further studies were made on packing dates under a vacuum, but filling with nitrogen before pasteurization. Results of these studies indicated that both the color and texture of dates can be successfully preserved during and after pasteurization by processing in a vacuumized container and filling the vacuum with nitrogen or other inert gas prior to hermetically sealing, and then pasteurizing.

Dates used in these experiments were the Deglet Noor variety grown in the Coachella Valley of California. This date variety averages 20 to 25 percent moisture content and contains little or no invert sugar. Its annual production amounts to about 25 million pounds per year, and production is still increasing. Work is also under way on the canning and pasteurizing of the highly perishable soft, invert-sugar-containing type of date grown in the Salt River Valley around Phoenix, Ariz. Such varieties include the Saidi, Khadrawi, and similar Persian dates. The annual domestic production of these dates now amounts to about 5 million pounds per year.

Low-Moisture Powders Made From Dried Prunes and Figs

A study of various drying procedures that might be used to prepare low-moisture powders from commercially dried prunes and figs and a comparison of the powders prepared by the different methods was almost completed by the Western Regional Laboratory. Smaller sizes of prunes and figs, which are not readily marketed through regular channels, were found to be suitable for conversion to low-moisture edible powders.

In cooperation with the California Prune and Apricot Growers' Association, acceptable prune powders have been prepared by drum, vacuum, and spray drying. Excellent powders were prepared by drum drying, and the continuous process made possible by this method of drying (which involves steaming, pureeing, drum drying, and grinding) makes this procedure economically attractive. However, special conditions might favor the less economical vacuum drying or spray drying in certain instances.

Powders were also prepared from several varieties of commercially dried figs. Satisfactory products were obtained by vacuum, drum, or air drying, following by grinding. This work was conducted in cooperation with the California Fig Institute, whose grower members have long been seeking new outlets for their fruit, particularly for the Black Mission variety. The investigation showed that the Black Mission fig is quite suitable for the production of powders.

Among various uses suggested for fig and prune powders, incorporation in baked goods and breakfast cereals seems most promising. On the basis of experimental bakery tests, it appears that addition of about 1 percent of fig powder to whole-wheat dough yields bread having improved flavor and color. For this purpose, powder prepared from small sizes of Black Mission figs was satisfactory and slightly superior to that prepared from figs of the Adriatic variety. Addition of fig or prune powder to the extent of 6 percent of the mixture yielded, after extrusion and drying, a granular wheat breakfast cereal of pleasing flavor and texture. It seems likely that fig powder would prove convenient for use in making soft fillings for fruit bars and other pastries. The admixture of such fruit powders to sugar and a gelling agent for preparing "instant desserts" has been suggested.

New Food Products Made From Fresh Prunes

Attractive new food products, made from freshly harvested prune plums at the Western Regional Research Laboratory, are being con-

sidered for commercial development by the prune industry. A puree, a cloudy juice, a clear juice, and a juice concentrate appear to have possibilities for use in beverages, baby foods, pharmaceuticals, and formulated foods, thus broadening the market for this fruit. The demand for dried prunes has been declining over a period of many years, so that surpluses are frequent and serious. The new products have a flavor and color that suggest plums rather than prunes.

Except for a few preliminary experiments based on published information, experimental production of the juices and purees was carried out on a pilot-plant scale. Orchard-run prunes of the French variety, harvested at about midseason, were used. Sorting was limited to the removal of obviously unound fruit. All the samples were heat-processed and packed in glass, except for a few that were packed in tin cans for comparing keeping quality in the different containers.

On the basis of results obtained in the pilot-scale tests, the following procedures have been proposed for producing the various products. Prunes are washed in a conventional washer-elevator. Sorting is done on a succeeding inspection belt. The prunes are then heated to inactive oxidative enzymes in a screw-type preheater into which live steam is admitted. A conventional pulper, fitted with appropriate paddles or brushes, is used to remove the pits. The puree is cooled to 100° F. by a heat exchanger, introduced into storage tanks and depectinized, and then pressed to separate the juice from the pulp.

For a cloudy product, the juice is flash-pasteurized, bottled hot, capped, and cooled. Clarification (for the preparation of clear juice) requires the use of a filter press after admixture of filter aid. A conventional, single-effect, vacuum pan is adequate for the concentration step, according to the processing tests.

Fruit-Cannery Wastes Processed Into Valuable Products

Bureau laboratories have made important progress in two distinct lines of investigation designed to find ways of solving or ameliorating the serious waste-disposal problem of the fruit-processing industry.

Feed from cannery waste

One approach to the fruit-cannery-waste problem is the conversion of the waste into stable products suitable for feeds and other marketable nonfood products. During the past year marked progress was made on the processing of cannery waste for this purpose. Pilot-plant studies were carried out on a process for making molasses and dried pomace from cannery waste comprising mixed pear and peach wastes, predominantly the former. This work was done by the Western Regional Research Laboratory in cooperation with the Cannery League of California in a pilot plant located near San Jose, Calif. The League supplied the necessary plant facilities, operating management, and financial support for the project. The Cling Peach Advisory Board, which represents both peach growers and peach canners, also participated in financial support of the project. The Bureau assumed responsibility for all technical phases of the operation, furnished the necessary technical personnel for the pilot-plant operation, and carried out process-development studies preliminary to pilot-plant experiments.

In view of the large amount of waste involved (about 75,000 tons in the San Jose area during the 1949 season), it was decided at the outset that any practical utilization process must handle all the waste as it comes from the cannery. This precludes the possibility of utilization for food and limits the product outlets to feed and other nonfood uses. The process as finally worked out in the pilot plant involved milling of whole pear waste, adding supplemental fiber to facilitate subsequent pressing operations, and treating the mixture with lime under carefully controlled conditions. After being aged, the treated material was pressed directly in a double-cone, continuous press. A number of different fiber supplements, including chopped alfalfa, lettuce meal and classified dried-pear pomace, gave satisfactory results.

No serious difficulty was encountered in handling the wastes from Bartlett, Hardy, and Bosc pears of a wide range of maturity and containing in some cases large amounts of grape, tomato, and peach wastes. One ton of typical pear-cannery waste yielded about 110 pounds of pear pomace containing 8 percent of moisture and about 220 pounds of pear molasses containing 75 percent of soluble solids.

Observations made during the season indicated that partial de-juicing of the treated waste before pressing is feasible and that marked improvement in juice clarity and press capacity will result from this intermediate juice separation. Laboratory studies are under way to provide the information needed for the indicated process improvements. Preliminary results of these studies indicated that substantially better operation can be effected during the further pilot-plant work planned for the 1950 pear season. Thereafter, commercial application of the process will probably depend only on the results of product evaluation studies now in progress. As a part of the product evaluation, feeding tests on the materials produced in the pilot plant are being carried out cooperatively with the California State Agricultural Experiment Station. Tentative cost estimates indicated that the probable value of pomace and molasses products from the utilization plant would be sufficient to make the operation less costly to the canners than any acceptable straight disposal method.

Sugar recovery from pear waste

A second promising approach to the pear-canning industry's waste problem is the recovery of sugars from waste peelings and cores by a process under development at the Bureau's Fruit and Vegetable Products Laboratory in Pullman, Wash. Sugars and other soluble constituents of pear waste are recovered in the form of juice, which is then purified to a clear colorless liquid that the canner may use as a sirup in canning pears. Refined sugar is added as needed to produce the desired degree of sweetness. Sugars, in the form of sirup, recovered from pear peelings and cores by this process would replace about one-third of the refined sugar now used in canning pears. Preliminary canning trials indicated that use of the juice sirup results in canned pears of improved flavor.

Difficulty in separating sugars and other soluble constituents from pear waste has been largely responsible for the failure to develop useful products from this waste. This problem was solved by the development of a lime-gelation process which converts the milled waste into a calcium-pectinate gel. This gel can be easily pressed to

yield a clear brown juice and a dry press cake from which the press cloths can be easily stripped. The gel is produced by adding lime or whiting at the rate of from 2 to 7 pounds per ton of milled waste and permitting the mass to cure for about 1 hour.

Several treatments are necessary to purify juice obtained by the lime-gelation process into a clear, water-white liquid of proper acidity for use as a canning sirup. The calcium dissolved by the juice during the lime-gelation process is removed by means of an ion-exchange resin. This treatment also increases the acidity of the juice to the desired point. Color is removed by treating the boiling juice with gelatin and a decolorizing carbon and filtering.

These investigations will be continued in cooperation with the Hood River Apple Growers, a farm cooperative located in Hood River, Oreg., for the purpose of translating the laboratory process into a semicontinuous pilot-plant operation. Improvements in processing operations and accurate cost estimates will be sought. A small commercial pack of pears in juice sirup will be prepared for use in making consumer-acceptance tests of this product.

Wax Treatment for Picking Boxes Retards Mold in Berries

An improved wax-treating method was developed to help combat the problem of mold growth on wooden berry-picking boxes (commonly called hallocks) used for transporting berries from the field to the processing plant. The problem is a serious one, especially in the Pacific Northwest. Sound berries put into mold-infected boxes frequently undergo severe damage before they reach the processing line and must be discarded. The financial loss resulting from damage to the berries and the need for frequent replacement of the hallocks is a matter of concern to both growers and packers. Impregnation of the wooden boxes with wax to prevent absorption of berry juices has been recognized as a possible solution to the problem. A few processors have attempted to accomplish this by treating the hallocks with paraffin wax. However, the method has not found general acceptance in the industry, because, too frequently, waxes of poor physical characteristics were used, and treating practices were poor; consequently the resulting coatings were of inferior quality and did not effectively inhibit juice absorption and mold growth.

In response to numerous requests from berry growers and processors, the Western Regional Laboratory undertook to find a solution for the problem. The best immediate answer appeared to lie in determining optimum wax characteristics and establishing definite treating procedures to insure a coating of maximum protective value. Laboratory studies on a large number of wax mixtures over a wide range of treating conditions indicated that the best treatment involves dipping the air-dry hallocks in a molten mixture of 95 percent paraffin wax (M. P. 143°-150° F.) and 5 percent microcrystalline wax (M. P. 160°-165° F.) under controlled conditions of temperature and time. Close observance of correct treating conditions is important. A simple spot test on the treated hallocks shows whether the waxing is being properly done.

Mimeographed circulars (AIC-239 and AIC-270), describing the wax-treating method and suggesting designs for treating equipment,

were prepared and distributed to commercial berry growers and processors. The considerable interest shown in the method indicates that the recommended procedures will be widely adopted.

Properly waxed hallocks remain substantially free from mold growth if washed after each trip. Loss of fruit due to mold damage is minimized or entirely eliminated. In addition to the prevention of berry losses due to molding, a substantial improvement in pack quality can be expected wherever properly waxed hallocks are used instead of untreated hallocks. An actual saving in hallock cost may also result, since the treatment more than doubles the useful life of the hallocks.

MISCELLANEOUS FOOD PRODUCTS AND PROCESSES

Antibiotics Tested as Food Preservatives

Antibiotics are substances, synthesized by some plants or micro-organisms, that are able in extremely dilute solution to kill other micro-organisms. The best known antibiotic is, of course, penicillin. Use of antibiotics in medicine has received intense study, but the possibility of using them in preservation of food has heretofore received only meager attention. At the Western Regional Research Laboratory, preliminary surveys, made to determine the ability of different antibiotics to combat food-contaminating organisms, indicated that subtilin had promising properties.

Subtilin is an antibiotic that was identified and isolated at the Western Laboratory. It is produced in cultures of a particular strain of *Bacillus subtilis*, the "hay" bacillus. Subtilin can be made either by surface or submerged fermentation procedures. Methods for its production in maximum yield and for its separation and purification have been studied in detail. Briefly, subtilin solution is obtained by extraction of the culture broth with butanol. The antibiotic is then thrown out of solution by the addition of salt and purified by several further extraction and precipitation steps. Chemical studies indicate that subtilin is a polypeptide and that it is destroyed, at least in part, by the digestive enzymes in the body.

In concentrations of only a few parts per million, subtilin was found to inhibit the growth of several food-contaminating micro-organisms. Particular micro-organisms not attacked by subtilin were those readily destroyed by heat. Conversely the more heat-stable forms were sensitive to subtilin.

Canned packs of uninoculated vegetables and other foods were prepared, and subtilin was added to them in low concentrations. The cans were heated so that the contents were above 200° F. for a number of minutes—sufficient heat treatment to kill the heat-sensitive bacteria, yeasts, and molds, but considerably less than required in usual canning procedures. It was found that such experimental packs were stable for many months and that the flavor and texture of several products—among them corn, broccoli, cauliflower, and brussels sprouts—were much better than those obtained when the cans were cooked for longer periods.

These results were promising enough to stimulate more concentrated effort in this field of work. Experiments were started to determine

whether subtilin would be safe as a food additive. The results of this work are reported under "Toxicological and Pharmacological Tests Made on New Products," page 95. They indicate that no harm results from oral administration of subtilin in amounts up to 0.1 percent of the diet of rats. Studies were begun to determine the mode of action of subtilin on bacterial spores. In general, it was found that the combination of heat treatment and subtilin was required and that suppression of the spores was not immediate but occurred when they began to germinate. Information concerning the factors responsible for spore germination is meager; hence work has been started to broaden this field of inquiry.

Other experimental packs showed that subtilin could not suppress large numbers of added spores. For example, some packs of food, to which bacteria spores had been added, spoiled in spite of the presence of subtilin. Studies to determine the causes of these failures are in progress.

Whole-Egg Powders Greatly Improved

Dried eggs, or powdered eggs as they are frequently called, were held in poor regard by many members of the Armed Forces during the late war. This was largely due to deteriorative changes that occurred in the dried eggs during storage and transportation. By the time these eggs were prepared for scrambling, the development of off-flavors was quite pronounced, and the reconstituted product often had poor texture. Certain improvements in the storage life of whole-egg powders were achieved toward the end of the war by means of modified processing and packaging methods. One such method was developed at the Western Regional Research Laboratory, which cooperated in the Government's emergency food-dehydration program. This was the preacidification process mentioned in the 1946 report.

It was recognized that there is need for even further improvement; therefore the fundamental study of dried-egg deterioration was resumed under an RMA project in 1948. This study has the long-range objective of developing a powder of improved stability for use in ready-mixed baking products, e. g., cake mixes, as well as for use by military forces and institutions. For use in ready-mixed baking products, the binding action of the egg must be conserved.

The first approach to the problem had as its immediate objective the identification of the chemical reactions responsible for deterioration in storage. Results obtained thus far have shown that the small amount of glucose normally present in egg (1.2 percent of the total solids) contributes more to this deterioration than was heretofore supposed. It has been demonstrated that the glucose reacts during storage not only with the egg proteins, as previously recognized, but also with cephalin, a phospholipid contained in the fatty fraction of the egg. The latter reaction has been shown to involve the aldehydic group of glucose and the primary amino group of the cephalin and appears to be a principal cause of off-flavor development in the dried egg.

Glucose-free egg powders have been prepared both on a laboratory scale by freeze-drying and on a commercial scale by spray-drying liquid egg after yeast fermentation to remove the sugar. Bakers'

yeast was used to remove the glucose in a 2-hour, resting-cell fermentation. Growth of contaminating micro-organisms during this period is negligible. The initial quality of the commercially prepared glucose-free egg powder is at least as high as that of the normal product. Moreover, the glucose-free powder is markedly more stable than a product containing glucose as regards both flavor deterioration and loss of baking quality. A glucose-free powder, containing 2 percent moisture and packed in contact with nitrogen instead of air, was found to suffer no measurable flavor deterioration, even after 2 months' storage at 100° F., while an egg powder containing glucose under similar conditions was considered unfit for table use after 1 month. Differences in the retention of baking quality, as measured by sponge-cake volume, were equally striking. A glucose-free egg powder containing 5 percent moisture and stored 6 months at 100° F. made an acceptable cake, while an egg powder containing glucose stored and used under identical conditions did not; in fact the batter made with an egg powder containing glucose failed to rise at all.

The chemical and physical tests commonly used to evaluate egg powders have been shown to actually measure the extent of reactions promoted by glucose. Thus glucose is directly involved in browning, decrease in pH, decrease in potassium chloride-soluble protein, increase in salt-soluble and ether-soluble fluorescence, and increase in creaming of ordinary dried whole egg during storage, and all of these changes are practically eliminated by glucose removal before drying. The preacidification procedure, described in the 1946 report, has been found to retard, but does not eliminate, these changes. Other deteriorative reactions, not involving glucose, are more readily demonstrated and can be more advantageously studied with the glucose-free powders. None of the usual chemical tests are useful for detecting oxidative deterioration in ordinary dried eggs. However, since the glucose damage can be readily prevented by the simple expedient of removing glucose, present plans call for a study of the mechanism of oxidative deterioration and other types of deterioration in glucose-free egg powder and means for producing egg powders of even greater stability.

Microbiological Investigations Continued on Poultry Products

The Microbiology Research Division's process for rapid, pure-culture fermentation of egg white by resting-cell suspensions of *Streptococci*, which was mentioned in last year's report, has been evaluated on a laboratory scale. The fermentations were successfully carried out on large volumes of fresh egg white. *Streptococcus lactis*, as well as other streptococcal species, were used in the process, which required 1 to 4 hours for completion at temperatures ranging from 21° to 45° C. There was no significant proteolysis and no proliferation of contaminating organisms. Egg white varying in final pH from 5.6 to 6.5 was produced as desired. Fermented white, produced in this way and subsequently dried, was used in baking angel-food cakes that were judged to be excellent by independent taste panels. Negotiations are now under way for evaluation of the fermentation process under pilot-plant and commercial conditions.

In continued studies on the effectiveness of various antibiotics against the *Salmonella* (food-poisoning) organisms found in egg powders, the mode of action, minimal inhibitory dose, influence of inoculum size, and development of resistance to antibiotics (aureomycin, chloromycetin, and polymyxin B sulfate) have been determined in test tubes for three *Salmonella* types by the tube-dilution technique. Data have also been obtained by similar tests on the antibiotic activity of polymyxin B sulfate against 32 *Salmonella* strains representing several different types.

In tests with developing chick embryos, the minimal lethal dose was determined for three *Salmonella* types. The effectiveness of the three antibiotics previously mentioned was also investigated by this same technique.

In cooperation with the Microbiological Institute of the National Institutes of Health, preliminary experiments on newly hatched chicks have been undertaken to determine both the infectivity of certain *Salmonella* strains, isolated from egg powder, and the effectiveness of the aforementioned antibiotics for preventing these infections.

Under a Research and Marketing Act project, a study was made of possible occurrence and significance of pathogenic organisms in frozen foods. Emphasis was directed toward precooked frozen foods, although the study was not limited to this type of product. Samples of frozen chicken chow mein, chicken a la king, and chicken pot pie, purchased on the open market, are being examined for microbial content. Wide variations in quality have been observed thus far.

Research was continued under another Research and Marketing Act project both by the Bureau's Microbiology Research Division and by nongovernment institutions under RMA contracts. Studies on the development of improved methods for the isolation of *Salmonella* organisms from egg powders have shown that lithium chloride in certain concentrations appears to be effective when incorporated in enrichment media. Comparative evaluation by known methods is still being made; however, with the knowledge obtained from studies on enrichment and selective media for the isolation of *Salmonella*, new methods are being developed for estimating the numbers of *Salmonella* organisms per unit quantity of egg powder.

Continued studies on the nutritional requirements of *Salmonella* organisms have been directed especially toward determining the minimal and maximal concentrations of energy sources utilized for growth by two strains of *Salmonella*. With each one of several organic substances, including DL-alpha-alanine, asparagine, glycerol, lactic acid, and sodium citrate, it was found that, generally, growth increases directly with increase in concentration of the energy source in the concentration range of 0.005 to 1 percent. Definite maximal concentration levels for the test substances were difficult to attain. The application of these findings to the preparation of synthetic media for propagating *Salmonella* organisms is being considered.

Progress has been made in the investigation to determine if *Salmonella* strains isolated from egg powder are harmful to human beings. This work is being conducted by a nongovernment agency under an RMA contract. Three strains each of three *Salmonella* types have been fed to groups of human volunteers under controlled conditions. Data are accumulating on the course of activity, but in long-term

experiments of this kind interpretations of the results must be deferred until the experiments are completed.

Another contractual phase of the same RMA project was undertaken to obtain information that would contribute to improvement in the microbial quality of poultry products. This investigation, started only recently, is directed toward learning where and how food-poisoning types of bacteria enter poultry products at the various stages in the processing line.

New Food Products Made From Rice

Several new food products, which may have practical importance, have been made from rice under an RMA project at the Western Regional Research Laboratory. One of them, rice curls, has been offered to industry and has attracted widespread interest. Rice curls are a snack food that can be made from low-priced grades (containing broken grains) of either California or southern varieties of rice. A coarse rice grit, prepared by grinding, is made into a stiff paste with boiling water and extruded as strings into hot cooking oil. Flavoring is optional, but a mixture of salt and monosodium glutamate is recommended. Cost studies of the process indicated that the manufacture of rice curls is suitable for a small business, since only a moderate outlay of capital is required for equipment. Informal consumer-acceptance tests in small groups indicated that rice curls are preferred to other products in the same general class by many persons.

Rice puffs are also a new product. They are made by immersing commercial parboiled rice in fat at 200° C. or exposing such rice to a current of air preheated to 250° C. The normal moisture content of parboiled rice (8 to 14 percent) is suitable for puffing the rice in oil, but, for puffing in hot air, the moisture content of the rice should not exceed 8 percent. The puffed product is suitable for out-of-hand eating, for breakfast cereal, or for making into confections with candy sirups or coating chocolate.

Considerable study has been given to the development of frozen cooked rice on the assumption that home-cooked rice lacks popularity because most housewives find it difficult to prepare properly. With the assistance of a taste panel, best cooking procedures for both long-grain and short-grain rices were first worked out. The rice was then subjected to frozen storage in cellophane bags and evaluated by the taste panel at intervals. It was demonstrated that no detectable deterioration occurred at 10° F. in 8 months, and that, instead, there seemed to be a slight improvement in color and texture over freshly boiled rice. Cost studies on this product are in progress. A problem remaining to be solved is that of mechanical packaging before freezing, because of the nature of the material. The preparation of frozen cooked rice is being extended to brown and parboiled rices, and also to rices of long- and short-grain types.

In accordance with recommendations of the Rice Advisory Committee, high priority is being given to the development of a satisfactory quick cooking rice. Tentative standards set up for such a product are as follows: The product must be one that can be made from either long-grain or short-grain rice; it must be dry and suitable for packaging in nonmetallic containers; and it must be rated by the

taste panel as equal to well-cooked, freshly prepared rice of the same grain type.

A superior product has been made from long-grain rice (Texas Patna) and almost meets these standards. A limited number of taste tests indicated that the texture of the product softened in boiling water is only slightly inferior to that of freshly cooked rice. The steps used to produce the quick-cooking rice involve soaking in water for 30 minutes, boiling for 2 minutes, puffing in a stream of air for 1 minute at about 200° C., and finally drying for 1 minute by lowering the air temperature to about 135°. This product can be made into a very choice cooked rice by softening in boiling water for 5 minutes.

TANNIN-BEARING PLANTS

Potential Domestic Tannin Sources Investigated

Investigations of various domestic sources of tannins potentially available have been continued at the Eastern Regional Research Laboratory. These materials include sumac leaves, hemlock and oak barks, chinquapin wood and bark, saw palmetto stems and roots, and canaigre roots. The results of the work on canaigre under an RMA project are given under a separate heading.

Sumac

Studies of tannin contents and abundance of wild sumacs, which have extended over a number of years, have been completed. The data have been published in U. S. D. A. Technical Bulletin No. 986, Tannin Content and Other Characteristics of Native Sumac in Relation to Its Value as a Commercial Source of Tannin. Data obtained in an intensive survey of the sumac growing wild in an area of approximately 12,000 square miles in the southernmost tier of counties of Virginia indicate that about 43,000 long tons of dry sumac leaf would be available there annually.

A total of 1,039 samples were analyzed for tannin and related constituents. In general, leaves, leaflets, and flowers were high in tannin, and petioles, rachises, stems, bark, wood, and seed were low in tannin. The average tannin content of moisture-free leaves of each species was (in percent): *Rhus trilobata*, 39.14; *R. copallina*, 36.68, 33.09, and 27.05 (for the three geographic areas studied); *R. glabra*, 27.28; *R. typhina*, 25.60; *R. lanceolata*, 22.01; *R. aromatica*, 21.76 *R. virens*, 20.37; and *R. microphylla*, 19.32. The data indicate that *Rhus copallina*, *R. glabra*, and *R. typhina* are most promising for commercial development and that *R. trilobata* might prove of value under certain circumstances. Bulletin No. 986 gives data that will be of value in the studies of the cultivation of sumac, as it indicates locations where suitable seed or planting stock might be obtained and discusses the influence of certain genetic and environmental factors.

In cooperative studies with the Bureau of Plant Industry, Soils, and Agricultural Engineering on the development of high-quality planting stock, analyses have been completed on 83 samples of sumac leaves collected in August and September 1949 at Beltsville, Md. One sample of leaves obtained from *Rhus coriaria* plants, grown from seed obtained from Italy, contained only 23.2 percent tannin.

Hemlock bark

Considerable quantities of hemlock bark from slabs and edgings are wasted annually in the Upper Peninsula of Michigan. Salvage and conversion of this bark into tanning extract would furnish an additional domestic supply of tannin. To investigate this potential tannin supply, representative samples of this bark were obtained from the Michigan College of Mining and Technology for use in cooperative studies. Analyses made at the Eastern Regional Research Laboratory showed that these barks contained about 13 percent tannin and that purities of the extracts were about 70. A survey by the Forest Products Research Division of the College has indicated that the smallest extract plant that could be operated economically would require annually about 12,000 cords of bark and should produce about 1,000 tons of 100-percent tannin. It appears that a supply of bark adequate for the operation of an extract plant should be available, since 125,000 cords were reported as available in the Upper Peninsula in 1946. Arrangements have been made to study the mechanical separation of bark from wood in chipped slabs and edgings.

Oak bark

In cooperation with the Tennessee Valley Authority, studies are being conducted on oak barks recovered from slabs and edgings produced in lumbering operations in the Tennessee Valley. Representative samples of the various oaks were examined for tannin. On the basis of their prospective value as sources of tannin, the barks with their tannin contents and purities of extract have been placed in four groups: (1) Chestnut oak, 15.30 percent tannin, purity 70; (2) southern red oak, blackjack oak, and black oak with tannin ranging from 9.20 to 9.90 percent and purity from 61.20 to 68.61; (3) post oak, scarlet oak, and white oak with tannin ranging from 6.09 to 7.41 percent and purity from 56.08 to 61.39; and (4) northern red oak with tannin 5.40 percent and purity of 52.12. On the basis of tannin content and purity, the barks in groups 1 and 2 could be used for making tanning extract. Those in group 3 could be used in small proportions in mixtures with groups 1 and 2, but not advantageously alone. It has been estimated that about 50,000 tons of air-dry bark would be available annually in the area. This amount should be adequate for the operation of a tanning extract plant. Further studies of this problem are being considered.

Chinquapin

In further tests with the Ozark chinquapin, tanning extract has been prepared, and tanning tests have been conducted. The extract resembled chestnut wood extract; penetration was slow; and the leather produced was dark in color, but firm and well filled.

Saw palmetto (*Serenoa repens*)

The two samples of this material studied recently represent material growing above and below ground. They showed 8.4 and 8.3 percent tannin, 5.4 and 4.2 percent total sugars, and 13.9 and 17.9 percent starch, respectively. Extraction was difficult because of the starch, and extracted liquors were low in purity. The results confirmed

previous data in indicating that this material is not suitable for use in producing tanning extract.

Canaigre Being Developed as Source of Tannin

Work on the development of canaigre roots as a source of tannin is being conducted cooperatively as an RMA project by the Eastern Regional Research Laboratory and the Bureau of Plant Industry, Soils, and Agricultural Engineering. The latter does all field and production work, and the former conducts all laboratory and pilot-scale processing studies.

About 30 tons of fresh canaigre roots were harvested in 1949 from experimental plots at Yuma, Ariz. Fifteen tons were shipped to the Eastern Regional Research Laboratory for use in drying and leaching studies, and the remainder was used where grown to prepare 4 tons of dried, powdered, canaigre root.

Preliminary laboratory tests indicated that powdered canaigre root might be used to partly replace quebracho tanning extract in regulating the viscosity of oil-well drilling muds. Arrangements have been made with the Humble Oil & Refining Co. to test this material in actual oil-well drilling.

Shredded canaigre roots must be dried to prevent mold growth and spoilage. Tests to determine the maximum permissible moisture content showed that no mold developed on samples held at 73° F. and 76 percent relative humidity for 169 days when the moisture content of the roots did not exceed 15.7 percent.

Analytical and laboratory leaching tests on canaigre roots dried under 12 different conditions showed that shredded roots can be satisfactorily air-dried without spoilage or loss of tannin, either out of doors or indoors on concrete, when spread at the rate of 1 pound per square foot of surface.

To improve the purity of canaigre tanning extracts by removal of sugars, root shreds were preleached with cold water, then comminuted by wet pulping, and subsequently extracted with either water or a three-to-one mixture of water and acetone. On the basis of relative purity of the final extract, best results were obtained by five preleachings with water at 4°-5° C., followed by extraction with 25-percent acetone solution. This procedure resulted in purities above 90, but more than 13.4 percent of the total tannin was lost in preleaching. With one cold-water preleaching at 1°-3° C., followed by water extraction at a moderate temperature, an extract having the acceptable purity of 65 was obtained, and only 6.7 percent of the total tannin was lost in preleaching. Further modifications of this procedure will be studied.

Moisture-free canaigre roots contain from 25 to 40 percent starch, most of which remains in the residual material after the tannin has been extracted. Utilization of this starch by hydrolysis and fermentation to valuable byproducts is important for the economical development of canaigre.

In laboratory tests, a countercurrent system of hydrolysis, using 6-percent sulfuric acid, completely hydrolyzed the starch in extracted canaigre, producing a 9.3-percent sugar solution. Complete hydrolysis was also obtained by use of 3 percent of the amylase Rhozyme S.

Both of the hydrolyzates obtained were fermented readily with yeast, 90 percent or more of the sugar being consumed. Eight yeast cultures, tested to determine rapidity and extent of sugar utilization and amount of alcohol produced, gave satisfactory fermentations. Three of these cultures will be studied for use in a continuous process.

It was observed that canaigre extract possesses antibiotic properties, which are not due to canaigre tannin. Tests in the Biologically Active Chemical Compounds Division showed that canaigre extracts inhibit the growth of *Mycobacterium phlei*, which belongs to the same genus as the organisms that cause tuberculosis in animals. The antibiotic properties of canaigre are receiving further study.

Several heavy leathers of the sole-leather type were successfully tanned in the Laboratory with canaigre extracts alone and blends of canaigre extracts with other tanning extracts. The canaigre extracts were prepared in pilot-scale extraction studies at the Eastern Regional Laboratory. The leathers produced were firm and well filled, and the yields compared favorably with those obtained in commercial practice. The results showed that canaigre tanning extracts can be used to replace other vegetable tannins now employed in the tanning industry.

A more economical method for drying fresh canaigre was developed in chemical engineering studies. It includes comminuting the roots in a rotary-knife cutter or a sharp-knife hammer mill having $\frac{1}{4}$ - or $\frac{1}{2}$ -inch screens and drying in a direct-heat, rotary, kiln drier. Air at 500°-600° F. inlet temperature was used without lowering the tannin recovery when the material was subsequently leached after being reground.

A commercially available, continuous, countercurrent extractor was modified for pilot-plant-scale leaching studies. The many variables involved in continuous, countercurrent extraction were studied in 88 leaching runs, each of at least 8 hours' duration. For most of these runs, the dried roots were ground in rotary, cylindrical-knife cutters equipped with $\frac{1}{8}$ -inch or $\frac{1}{16}$ -inch screens. The solvents used included distilled water, 10-percent and 18-percent aqueous isopropyl alcohol, and 25-percent aqueous acetone. Liquids-to-solids ratios ranged from 4 to 25, temperatures from 80° to 175° F., retention times from 1 to 5 hours, and the number of extraction cells used from 8 to 14.

The results obtained suggested the following as suitable conditions for the large-scale extraction of canaigre. With the modified extractor as described, ground, dried roots may be extracted (1) with aqueous organic solvents, using a liquid-to-solids ratio of 10 to 1 and a retention time of 90 minutes in a 10-cell extractor; or (2) with water, using a liquid-to-solids ratio of 10 to 1 and a retention time of 120 minutes in a 14-cell extractor. Under these conditions, when 18-percent aqueous isopropyl alcohol was used at 112° F. actual tannin recoveries up to 94 percent of the total tannin were obtained. With distilled water at 112°, and the state of subdivision described, the recoveries were 73 percent of the total tannin, but at higher temperatures recoveries up to 78 percent were obtained.

Other pilot-plant studies have demonstrated that, by using feed concentrations of 25 to 50 percent solids and inlet-air temperatures

of 400° to 600° F., canaigre tanning extract can be spray dried to a satisfactory dry powder.

Preliminary cost estimates have shown that the continuous counter-current extraction of canaigre is the most feasible for commercial operation of any leaching process thus far studied for the recovery of tannin from this material.

HIDES AND LEATHER

Improved Insole Leather Obtained by Alum Retannage

In a continuation of the studies on alum retannage of vegetable-tanned leather for production of more serviceable insoles, five pilot-scale retanning tests were made at the Eastern Regional Laboratory. Twenty-five bellies that had been vegetable-tanned by a commercial tanner were used in each test. The results indicated that it should be possible to adapt the process to commercial operation.

In cooperation with a large tanning company, the alum retannage procedures were modified to adapt them to tannery conditions and schedules. Seven plant-scale tests were run in which the retannage was applied during the oiling of the leather in drums. For each drum-load test, 200 vegetable-tanned bellies were used. In the laboratory pilot-scale tests, the retanning mixture was added as a solution and was well distributed on and throughout the leather. In the tannery tests, the retanning mixture was applied as a concentrated solution in some cases, and as a dry mixture in others.

In the first tannery tests, the retanning mixture was a concentrated solution of aluminum sulfate which, in order to tan, required basicifying with sodium carbonate and stabilizing with sodium acetate. Use of this tannage in the oil drums, where all the salts were taken up by the leather, resulted in too much mineral salts in the leather, which made it tend to have a cracky grain. To overcome this difficulty, in subsequent tests the aluminum sulfate and the basicifying and stabilizing agents were replaced by a water-soluble basic aluminum acetate. This lowered the salts content of the finished leather and gave an increased fixation of aluminum oxide.

From the standpoint of tannery practice, it seemed desirable to add aluminum acetate as a dry powder to the wet leather in the oil drum, thus making it possible for the leather to absorb completely the sugar, salts, oils, and retanning mixture and leave no excess of liquid in the drum or on the leather surface. In some cases, however, this procedure distributed the tanning mixture nonuniformly throughout the drum load of leather, and thus resulted in nonuniform retannage.

The best retannage results were obtained at the tannery when a solution of aluminum acetate was added to the wet leather after it had been loaded into the drum. The drum was then run for a short time before the filler materials were added, and again before and after the oil-sugar mixture was added. The usual addition of Epsom salts was omitted.

The leather obtained by this procedure showed a fixed-aluminum-oxide content of 3.5 percent, a pH of 4.43, and a shrinkage temperature above 116° C. When immersed in boiling water for 3 minutes, the leather showed no hardening or area shrinkage. Examination of the

leather, split into seven thin layers, showed that the alum retannage had penetrated well throughout the leather, the middle layer containing 2.54 percent of aluminum oxide.

Some of this leather has been used for insoles in the manufacture of shoes which are now being subjected to service tests. Samples of the same leather are being subjected to physical deterioration tests in the laboratory.

Two manufacturers of belting leather, who are interested in the possible advantage of retanning such leather with alum, have cooperated in some preliminary retanning tests in the laboratory.

ANIMAL FATS, DERIVATIVES, AND PRODUCTS

Improved Plastic Materials Made From Fats

Polymerization and copolymerization experiments with vinyl esters of the fatty acids have been continued and expanded by the Eastern Regional Laboratory. Polymerization techniques have been developed which permit the use of the commercially useful bulk, solution, emulsion, and pearl methods. Use of the vinyl fat derivatives permits the preparation of polymers that have a wide range of properties and will retain any plasticizer.

Polymers made with these fat derivatives have potential usefulness in important commercial products such as films, sheets, coating materials, adhesives, and rubberlike substances. Because of their improved physical properties, these new plastic materials have attracted considerable interest from industries.

Infrared-Absorption Studies Aid Industrial Utilization of Fats

The four most important industrial processes for converting fats to more useful commodities are hydrogenation, isomerization, oxidation, and polymerization. One of the greatest handicaps to further improvements in these processes, through research, has been the difficulty of analyzing the reaction products. For example, the best methods available for studying the formation of iso-oleic acid during hydrogenation has required several days for completion; moreover, even then the results obtained were of doubtful validity. By infrared-absorption techniques, developed at the Eastern Regional Research Laboratory, the percentage of iso-oleic acid in a hydrogenated fat can now be determined quickly and precisely, making possible a study of the formation of this acid during hydrogenation. The significance of this lies in the fact that formation of iso-oleic acid plays such an important role in the smoothness, creaming power, and hardness of the resulting shortening.

Infrared methods of analysis also are expediting studies on the oxidation of fat constituents directly to products having many potential uses in the preparation of high-quality plasticizers, protective coatings, and synthetic lubricants.

Optical Method of Fat Analysis Made More Accurate by Isolation of Pure Fat Acids

Greater accuracy in the spectrophotometric method of analyzing fats has been made possible through the successful preparation of pure natural linoleic, linolenic, and arachidonic acids at the Eastern Regional Research Laboratory. The spectrophotometric method for analyzing fats, as developed in this Bureau, has become the most valuable and precise method in this field and is extensively used in both research and specification-control work. It has been adopted as a tentative method of the American Oil Chemists' Society.

The properties of purified arachidonic acid, including the important spectral characteristics, were reported this year. Arachidonic acid is an important and characteristic constituent of animal fats and one of the essential fatty acids in human nutrition.

WOOL AND WOOL GREASE

New Process for Scouring Wool Uses Suint Salts to Advantage

There is urgent need for improved means of scouring raw or grease wool with recovery of byproducts. Compared with other industrial processes, the methods of cleaning wool have improved very little as the result of modern research. Although relatively little wool is scoured by dry cleaning, this method does permit ready recovery of the grease. However, the returns from sale of recovered grease have not sufficiently compensated for solvent losses and distillation costs. Accordingly most of the woolen mills still scour by the old procedure of scrubbing in a series of tubs containing soap and soda in water.

The process is slow, and the wool is subject to chemical damage by the alkaline solution. The most serious objection is the necessity of effluent disposal. Although methods have been devised for recovering grease from such effluents, these are so expensive that, for the most part, the mills have preferred to dump the wash water containing the grease directly into streams. The pollution resulting mostly from the presence of grease has been serious, and, with the present trend toward enforcement of legislation prohibiting stream pollution, the mills are faced with the necessity of devising an economical combination of wool cleaning, waste clarification, and byproduct recovery. Under present conditions in the textile industry, any additional cost incurred in the processing of wool would tend to weaken the position of wool in competition with other fibers.

The development of improved methods of scouring wool is one of the objectives of the wool research under an RMA project at the Western Regional Research Laboratory. The problem has been approached through fundamental studies of the mechanism of scouring with the ultimate aim of finding the best over-all combination of cleansing method, waste disposal, and grease recovery commensurate with minimum damage to the fiber.

Consideration of the fact that raw wool contains basic salts suggested that this problem might be solved through utilization of the naturally occurring suint salts in grease wool instead of soap for cleaning the

wool. The suint salts are perspiration residues and comprise an average of about 20 percent of the weight of grease wool. These salts, with added water, are effective in emulsifying wool grease, but the mixture of suint salts with water alone is not efficient for scouring.

Investigation established that scouring by suint salts and water can be markedly improved by adding relatively small amounts of alcohol (any one of several kinds). Moreover, with a slight increase of alcohol concentration, the grease can be made to separate from the emulsion and to dissolve in the alcohol. Thus it appears possible to recover the grease in a relatively pure form.

A method of continuous scouring, based upon these findings, has been demonstrated successfully on a laboratory scale. Grease wool is conveyed through an opposing stream of water containing 3 percent of butyl alcohol. The alcohol and suint salts effectively emulsify the grease. The grease content of the wool, which is normally about 12 percent before scouring, is reduced to less than 1 percent, which is the commercially acceptable high limit. If alcohol were not used, the mixture of suint and water would reduce the residual grease content only to about 8 percent.

To recover the grease, the butyl alcohol concentration of the scouring liquor is raised to around 7 percent. The additional alcohol breaks the grease emulsion and results in the formation of two layers, the upper layer containing alcohol and grease and the lower (aqueous) layer containing the dissolved salts and some of the alcohol. The grease is easily recovered from the decanted upper layer by evaporation of the alcohol.

An advantage of the method lies in the fact that only the 4 percent of added alcohol requires recovery. This is the amount of alcohol used to cause separation of the grease emulsion. The other 3 percent remains in the aqueous layer, which is reused for scouring without distillation. In this process the wool fibers are not in contact with alkali; hence they suffer little or no damage. The grease is recovered in relatively pure condition, which should make separation into useful constituents comparatively easy. The suint salts are allowed to accumulate only to a predetermined concentration in the recirculating solution; the excess is recovered from a diverted portion of the aqueous layer and may be used as a source of cheap soap or potash salts. As compared with the conventional soap-soda process, this method of scouring is more rapid, and the equipment requires less space, since a relatively small volume of washing solution is used.

More laboratory work remains to be done before the process is ready for large-scale tests. Studies of such factors as rate of flow and temperature will be required, as well as experiments with a wide variety of wools.

Industrially Useful Products Separated From Wool Grease

Fractionation of wool grease by molecular distillation, reported previously, has been followed by fractional crystallization from solvents such as isopropyl alcohol, methylethyl ketone, and acetone. Continuing its research under an RMA project, the Eastern Regional Laboratory found that wool grease can be separated into two fractions, one being a liquid at room temperature and the other a solid. The liquid

fraction, which comprises more than half of the original grease, has a good viscosity index; that is, it shows relatively little change in viscosity with change in temperature. This indicates potential utility in several commercial uses for which this property is important, such as lubricants and marking paints.

None of the studies on wool grease has indicated any worthwhile concentration of cholesterol esters in any of the fractions obtained.

PROTEINS AND PROTEIN PRODUCTS

Large Protein Crystals Isolated From Milk

Large crystals of globulin protein have been prepared from milk whey at the Eastern Regional Research Laboratory during an investigation on the separation of milk proteins. Single crystals were sufficiently large to be weighed and analyzed by ordinary methods. Direct analysis showed that the protein crystals contain about equal parts of protein and water, whereas the previously accepted values, based on indirect analysis, indicated a water content of only 20 percent. These findings have resulted in a revision of the molecular weight of this milk globulin from 40,000 to 35,000.

Protein crystals are unique in that they permit small molecules, such as those of salts and sugars, to diffuse into them when the crystals are suspended in solutions containing such small molecules. However, the concentration of the small molecules in the water of the protein crystal is never equal to their concentration in the suspending medium. This fact has led to the division of the water of crystallization of proteins into two types, designated "free" water and "bound" water; the "free" water is available as a solvent for small molecules, whereas the "bound" water is not available. The relative amounts of these apparently different types of water vary widely, depending on the concentration of small molecules in the suspending liquid. This variation in composition of the protein crystal with its environment is directly related to the difference between the osmotic pressure of the crystal and that of the solution.

The results indicate that the division of the protein water of crystallization into two kinds of water in definite proportions is erroneous. A more probable view is that the water in the protein crystal is held by the protein with varying degrees of firmness and that polar groups in the protein molecule compete with the osmotic attraction of dissolved substances for this water.

The densities and compositions of milk-globulin crystals suspended in serum-albumin solutions were compared with similar measurements made on crystals suspended in sucrose solutions. The results showed that the serum albumin did not pass into the water of crystallization of the milk globulin, but that sucrose did pass into the protein crystal increasing its density.

These measurements on protein crystals are useful for interpreting the behavior of viruses in solution. Thus, the density of a virus protein, as determined by centrifugation in serum-albumin solutions, is about 1.14, indicating a water content of 66 percent; in sucrose solution, the density is 1.25, corresponding to a water content of only 25 percent. Since the milk-globulin crystals suspended in these

solutions gave similar density values, and sucrose penetrated the water of the protein crystal, the value for the water content of virus protein obtained in serum-albumin solutions is probably correct.

Research on Nicotine Leads to New Fundamental Knowledge on Proteins

A study of the photochemical oxidation of nicotine in the presence of methylene blue, begun by the Eastern Regional Laboratory in 1948, led to the discovery of new nicotine derivatives, including what may be considered a dimer of nicotine dioxide, which appeared to be identical with, or similar to, compounds of nicotine that another investigator had isolated from fermenting cigar tobacco. Further work during 1949 showed that this photochemical reaction could be applied to tertiary amines in general and was useful as a new tool for research on proteins and viruses. Studies were made on the photochemical oxidation of tryptophan and other amino acids, the relatively pure protein beta-lactoglobulin, and the viruses of human influenza and Newcastle disease of poultry. When the viruses were photo-oxidized completely, they were completely inactive, but had also lost their antigenic properties.

In order to obtain a better understanding of this photochemical reaction on biologically active proteins, the oxidation of crystalline lysozyme and beta-lactoglobulin in the presence of traces of methylene blue was investigated further this year.

Preliminary results indicated that there was a gradual decrease in the enzymatic activity of the lysozyme which could be correlated to a large degree with the decrease in histidine and to a smaller extent with the decrease in tryptophan and tyrosine. When lysozyme was photo-oxidized to the extent of two moles of oxygen per mole of enzyme, all the histidine disappeared, while the enzyme activity decreased 70 percent. Methionine concentration in the lysozyme was not affected, even after 16 moles of oxygen was absorbed. From the results it appears that in the lysozyme molecule histidine represents the major active group, that tryptophan is active to a lesser degree, and that tyrosine is of questionable or minor importance. Methionine is not active in the lysozyme molecule.

Similar experiments on crystalline beta-lactoglobulin yielded essentially similar results. Solubility studies on this protein during photo-oxidation showed that the decrease in solubility was parallel to the decrease in tryptophan.

On the basis of these experiments, it appears that irradiation of lysozyme and beta-lactoglobulin in the presence of methylene blue results first in the oxidation of histidine, followed by the oxidation of tryptophan (with some overlapping), while the oxidation of tyrosine proceeds at a much slower rate. Although methionine is highly reactive when isolated, this amino acid is very resistant to photo-oxidation when it is a part of the protein molecule.

The action of light on proteins in the presence of methylene blue represents a promising approach in relating the structure of biologically active proteins, such as enzymes, viruses, toxins, and antibiotics, to their activity and offers the possibility of modifying structures and activity in a systematic manner.

Waste Feathers Converted to Fertilizer Material by Simple Process

The development of industrial processing of poultry has resulted in the concentration of poultry-packing plants in comparatively small areas, and this trend is continuing. The economic and sanitary disposal of the feathers is a very serious problem, since they amount to more than 40,000 tons annually.

The feathers, as they come from the poultry, are wet and sometimes mixed with blood, manure, and other offal; unless disposed of promptly, they putrefy. Washing followed by drying gives a product that can be stored, but this additional processing is expensive; the product is bulky; and no adequate commercial outlet has been found for dry feathers.

It was realized that the solution of the bulk-feather-disposal problem calls for large outlets. To achieve utilization on a large scale, two conditions are essential—a cheap, simple process to convert the feathers into a stable product and a market capable of absorbing profitably all that can be produced. In cooperation with interested industrial groups, the problem has been attacked by the Western Regional Laboratory in a manner to meet these two conditions, and a probable solution has been found.

The cheap, simple process developed by this work consists in treating the whole unwashed wet-feather residue of the packing plant with steam under moderate pressure in a closed vessel equipped with an agitator. Dry-rendering cookers, commonly used in fat-rendering plants, have been found satisfactory. The steam is turned directly into the cooker containing the agitated charge of feathers, and a pressure of 40 to 60 pounds is maintained for 60 to 30 minutes. Upon release of the pressure, the mass is discharged, dried by passage through a revolving drum drier, and bagged. In this condition it is practically odorless and can be easily stored or shipped.

This calcined feather product finds a ready market as an organic-nitrogen-containing fertilizer material. It contains 12 to 15 percent nitrogen, about 1 percent of which is water-soluble and therefore immediately available to plants. The remainder is slowly released in the soil. In admixture with inorganic fertilizers, the feather product acts as a conditioner to inhibit caking.

The processing costs have been estimated to be about \$34 per ton on a 5-to-10-ton-per-day schedule, and the product has sold for \$60 to \$75 per ton. There is practically no processing loss; a ton of raw feathers (dry weight) produces a ton of the calcined material.

It is possible that other uses will be found for this product. For example, it has been found that when it is modified by alkali it is as effective as calcined hoof meal and other proteins in common use as a component of plaster to retard setting. Furthermore, the product modified by acid has been demonstrated to be effective as a foaming agent for the control of oil fires. Calcined feathers might find use as a bulk protein in mixed feeds, or as a filler and supplement in protein plywood adhesives. However, there is a present market for it as a fertilizer, and this outlet is large enough to absorb large quantities.

Previously, efforts had been made at the Western Regional Laboratory to develop products of higher value from waste feathers. For example, monofilament fibers and bristles had been prepared from

cleaned feathers on a laboratory scale. The bristles offered promise for use in brushes, and the fibers could find application in such places as inner linings for clothing.

PINE GUM, DERIVATIVES, AND PRODUCTS

New Pine-Gum Product, Maleo-Pimaric Acid, Distributed for Evaluation

For many years only two products, turpentine and rosin, have been obtained directly from pine gum. One approach to diversifying the outlets for this raw material is to produce new, useful chemicals directly from the crude gum, without first processing it to turpentine and rosin. An example of a product made in this way is the addition product of maleic anhydride and levopimaric acid, a new naval stores product described in the report for 1948. For convenience this new product has been named maleo-pimaric acid.

With funds provided for Research and Marketing Act investigations, an enlarged pilot plant was placed in operation during the year. In this equipment barrel lots of crude gum were treated to obtain more data on the process and additional quantities of maleo-pimaric acid for distribution to firms interested in evaluating its applications.

The new acid offers possibilities of commercial use in the preparation of plasticizers, resins, and emulsifying agents. Its availability for commercial evaluation was announced in February 1950. Within 4 months 151 samples of the pilot-plant product were requested by and distributed to chemical manufacturers, research laboratories, and universities throughout the United States. Requests for samples were received from Canada, Europe, and South America. Several of these groups afterward requested additional amounts of maleo-pimaric acid, an indication that their preliminary tests were promising.

Now that commercial samples of maleo-pimaric acid have been widely distributed, the indicated markets will be closely followed in an effort to establish this product as an outlet for gum naval stores.

Useful Peroxides and Resinous Polymers Made From Turpentine

Research has continued on developing new industrial outlets for turpentine. During the year the Naval Stores Research Division improved its methods for producing peroxides from turpentine derivatives, acquired additional information as to their practical utility, and prepared new resinous polymers.

In continuation of a previously mentioned project in cooperation with the Government Laboratories of the University of Akron, methods were developed for producing terpene peroxides of relatively high purity in good yields. These terpene peroxides were evaluated at Akron for use as catalysts in the emulsion polymerization of butadiene-styrene in the production of "cold rubber" (at 5° C.). Comparison of the peroxides obtained from turpentine with commercial catalysts indicated the suitability of the new peroxides for use in this field. Especially satisfactory was the oxidation product of pinane which seems to be superior to the most commonly used catalyst, cumene hydroperoxide, derived from petroleum. The favorable results ob-

tained with terpene peroxides justify future research to obtain products having the maximum peroxide content. Improved methods of preparing the peroxides are necessary to make the production of these terpene derivatives economically attractive. Additional laboratory-scale preparations must precede larger-scale production.

Although economical methods of producing peroxides from terpenes and evaluation of their physical and chemical properties should provide a new outlet for a considerable quantity of gum turpentine, this development, as stated last year, was inspired not only as a means of extending the uses of turpentine components but also to supply an alternate catalyst in cold rubber production as a resource in an emergency.

Although beta-pinene, which comprises about 30 percent of gum turpentine, is used commercially in the preparation of resinous polymers, it has been impossible heretofore to prepare satisfactory polymers from alpha-pinene, the principal constituent of turpentine. During the past year resinous polymers having a high melting point and improved properties were obtained in promising yield from alpha-pinene. This work is being continued to obtain resins in higher yields and with still higher melting points in order to meet the requirements of consuming industries.

Better Metal Resinates Provided for Varnish Industry

The reaction of metals or metal oxides with rosin forms compounds known as metal resinates. One use for certain metal resinates is as driers in the varnish industry. For this use they must have a fairly high metal content, a low acid number, and adequate solubility in varnish solvents. Recent competition from new types of varnish driers has caused the Naval Stores Research Division to intensify its efforts to improve commercial metal resinate driers. As a result, a new process was developed whereby improved resinates can be easily prepared with high metal content and greater solubility than are found in the present commercial resinates. This development should enable the naval stores industry to regain a portion of the varnish-drier market.

The new process involves the fusion of chemically modified rosin or of a modified rosin derivative in contact with a suitable metal compound. This is the only method known by which neutral metal resinates of a permanently fusible type can be prepared. In laboratory experiments, nearly every metal resinate of commercial importance was produced, usually having a higher metal content and better solubility in hydrocarbon solvents than when prepared by the conventional method. For example, manganese resinate can be prepared with 8 percent manganese as compared with only 3½ percent of this metal in the usual commercial product. The newly developed resinate also has better color and solubility. A disadvantage of the present commercial manganese resinates is that a sludge sometimes separates from its solutions on standing. Use of the modified rosin or rosin derivative gave solutions that were stored in the laboratory for over a year with no sludge formation.

This development should permit the inclusion of calcium, zinc, and magnesium resins in a much wider variety of varnish formulations than is possible with the present type of metal resins.

In addition to the expected usefulness of these products in the varnish industry, they may have value as fungicides. A number of fused metal resins produced from modified rosin and rosin derivatives are being evaluated as wood preservatives.

Direct Aid Given to Pine-Gum Processors

Through its Naval Stores Station at Olustee, Fla., the Bureau continued to introduce practical improvements and render technical assistance to pine-gum processors who are applying the Department's patented Olustee process of gum cleaning. Since the 27 central gum-cleaning plants which use this process handle nine-tenths of all the pine gum produced in the United States, technical assistance to these plants is also helpful to the gum farmers for whom the plants provide a cash market for crude pine gum.

Accessory gum-processing equipment, described in last year's report, has been modified and widely adopted by the gum naval stores industry for the production of low-acid turpentine to meet the ruling of the Commodity Credit Corporation that turpentine must have an acid number no higher than 0.5 to be eligible as collateral for a loan. Processors using the new accessory equipment, which station engineers call a vapor-velocity-reduction chamber, can readily reduce the acid number of their turpentine to well below this value. Advice on the installation and operation of the new equipment has been given to operators by the station's technologists after personal inspection of the plants.

Several plants, which have used the Olustee process but had difficulty in producing the highest quality of gum rosin required to withstand the prolonged heat treatment incident to varnish making, have adopted the recommendation of the station engineers to use a double water washing instead of the usual single washing of the crude gum. This double washing removes traces of impurities which adversely affect rosin for varnish.

By means of these and many other services the Naval Stores Station has aided the industry in maintaining its competitive position.

RUBBER SOURCES AND PRODUCTS

Guayule Investigated Further as Domestic Source of Natural Rubber

This Bureau is trying to develop new or improved methods for the recovery of rubber of uniformly high quality from guayule shrubs as part of a broad program of preparedness authorized under the Critical Materials Stock Piling Act of July 23, 1946, to meet emergency needs for natural rubber in the event of war. Research on this problem is in progress at the United States Natural Rubber Research Station at Salinas, Calif., in cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering.

Deresination as a means of improving rubber quality

Crude rubber as normally obtained from guayule contains 20 percent or more of resins, and because of this it is inferior in quality, lacking in uniformity, and consequently limited in usefulness. The removal of these resins offers promise of yielding a crude rubber that will approach Hevea (plantation) rubber in quality. Solvent extraction of guayule shrub prior to its pebble milling has been shown on a laboratory scale to be an effective means for insuring that the crude rubber will have a resin content of only 2 percent or less. Solvent extraction of the crude rubber obtained from unextracted shrub is another means for effecting deresination, but this method has not been considered very practicable because of the relatively impermeable structure and other unfavorable physical characteristics of the solid rubber particles recovered by pebble milling, which are commonly referred to as "worms."

Recent work has shown that the deresination of resinous rubber worms is an alternative and possibly a more economical procedure than deresination of whole shrub prior to pebble milling. Use of a resin solvent, such as methylethyl ketone, at elevated temperatures has been found to make possible the dehydration and extraction of resins from wet crude resinous rubber worms in essentially a one-step batch operation. A minimum of solvent is required when the extraction is conducted near the boiling point of the solvent, but deterioration of the rubber has been noted after such hot extraction. Satisfactory means for protecting the rubber have not as yet been found.

Sample lots of deresinated guayule rubber obtained by deresination of the shrub and by deresination of rubber worms were submitted to one of the major rubber manufacturers for test. The results indicated that the rubber from deresinated shrub was the better of the two. These samples of deresinated guayule rubber were regarded with favor by the rubber firm which conducted the tests, and the results of the tests definitely encourage further development work on deresination.

Improvement in milling of guayule shrub

Basic improvement has been made in the pebble-milling process for the recovery of rubber from guayule. This consists of the processing of lush or freshly harvested shrub in contrast to the conventional processing of shrub which has been "conditioned" by field exposure or by prolonged storage. Conditioning, which actually consists of partially drying the shrub before milling, was formerly considered essential in effecting coagulation of the rubber in the shrub to prevent loss as latex in the milling water. The new process effects complete coagulation of the latex by thorough crushing and hammer milling, regardless of the moisture content of the shrub, and eliminates the necessity for the storage and drying steps. This development was cited in last year's report, but further confirming evidence of its superiority has since been collected through a direct comparison of results obtained by the new process with those obtained by a conventional conditioning treatment and by the method of bale-retting, a process previously recommended as promising by the Emergency Rubber Project.

The new development of utilizing freshly harvested shrub not only simplifies processing, with accompanying decreases in costs of harvesting and handling, but also results in higher over-all yields, greater uniformity, and superiority in quality of the rubber. This development provides an excellent basis for further process improvements with particular reference to continuous milling for recovery of rubber and ultimate replacement of the cumbersome and inefficient pebble mill.

THERAPEUTIC AND OTHER BIOLOGICALLY ACTIVE SUBSTANCES

Studies Begun on Possible Plant Sources of Arthritis Remedies

Cortisone and ACTH are new words of dramatic import, because they are the names of biological compounds that relieve persons suffering from rheumatoid arthritis. Their cost and scarcity aggravate the clamor for more and better sources of these and other antiarthritic compounds. At present they are obtained from animal glands, but this source is costly, and the supply is hopelessly inadequate. The answer must be found in their synthesis from certain compounds that exist in plants.

Chemically, cortisone is a steroid of particular structure and configuration. Somewhat similar steroids occur in plants, and some of them have a structure close enough to that of the animal products to give hope that chemical modification into antiarthritic compounds is possible. Thus the plant world is being searched for steroids of the proper structure.

The Eastern Regional Research Laboratory is cooperating with the Bureau of Plant Industry, Soils, and Agricultural Engineering on this problem. The latter selects promising plants, usually wild, and sends them to the Laboratory for extraction and characterization of the steroids. Promising steroids are then sent to the National Institutes of Health for biological testing and for chemical modification.

Sapogenins—plant steroids of the type sought—occur in certain plants combined with sugars as saponins. The latter, whose name implies that they act like soap in making water foam, are extracted from the dried plant material, first with boiling 70-percent (ethyl) alcohol and then with 95-percent alcohol. The alcohol-soluble fractions are combined and freed from lipids, pigments, and other unwanted compounds by dilution with water and extraction with benzene. The saponins remaining in the alcohol are hydrolyzed with hydrochloric acid to liberate sapogenins. To avoid any effect of acid on the liberated sapogenins, benzene is continuously cycled through the hot solution to remove the sapogenins as fast as released. The benzene extract is next treated with alkali to remove still other impurities, after which it is evaporated to dryness. The sapogenins may or may not crystallize. The crude material is further purified by high-vacuum distillation in a pot still. The sapogenins sublime and crystallize in the condenser, leaving impurities behind. Recrystallization from acetone yields a mixture of pure sapogenins. The mixture is resolved by adsorption on alumina followed by elution with solvents of increasing polarity. The final crystalline steroids

are identified by melting point, specific rotation, X-ray diffraction pattern, and chemical properties.

This routine procedure is used in the examination of a large number of plant species from different areas and at different stages of growth. It is not only a screening procedure to determine which species are worthy of detailed study, but it is also a method for the qualitative and semiquantitative determination of plant sapogenins, and for the preparation of substantial samples for use in biological and chemical research.

Certain Micro-Organisms Found To Be Good Producers of Vitamin B₁₂

During the past 10 years the gradual replacement of meat and fish meals and solubles by soybean meal in practical poultry rations has been accompanied by increasing severity of a nutritional deficiency which could not be attributed to a lack of any of the known vitamins, amino acids, or mineral elements. The characteristic low hatchability of eggs from hens fed all-plant-protein rations and poor growth of the resulting chicks are now attributed to a deficiency of vitamin B₁₂, the recently discovered antipernicious-anemia vitamin. However, long before vitamin B₁₂ was announced and before its importance in chick nutrition was established, the mode of attack that was to yield a solution to the practical feeding problem had become evident.

Of particular importance was the finding that manure is a rich source of the deficient vitamin, but only if it is allowed to stand at temperatures suitable for microbiological activity. This suggested strongly that micro-organisms in the voided fecal matter synthesize the vitamin—an explanation consistent with the known ability of various micro-organisms to synthesize other vitamins of the B complex. It also suggested the possibility of producing the vitamin by growth of a suitable microbe on an industrial scale, as is done for production of riboflavin. This approach was adopted by various laboratories working independently.

Cooperative work on the problem was started in December 1947 by the Western Regional Research Laboratory and the Washington State Agricultural Experiment Station. Various micro-organisms were grown at the former institution and were then assayed by chick-feeding tests at the latter. It was soon found that certain bacteria of the genus *Bacillus* gave significant growth responses. The announcement by industrial laboratories at this time of microbiological assay methods for vitamin B₁₂ and the identification of vitamin B₁₂ as the chick-growth factor permitted a much more rapid and comprehensive search for suitable vitamin B₁₂-producing micro-organisms.

As a result of this search at the Western Regional Laboratory, a strain of *Bacillus megatherium* (designated as B-938) was isolated from a soil sample obtained from an abandoned chicken yard at Washington State College. This bacterium grows very rapidly under aeration, a propagation period of only 7 hours being required, in a nutritive medium containing only a fermentable sugar, ammonium nitrogen, and inorganic salts. Beet, cane, or citrus molasses provides a cheap and suitable source of sugar. The sugar is utilized for cell growth with a yield equivalent to 50 pounds of dry cells per 100 pounds of

sugar. Seven milligrams of vitamin B₁₂ are contained in each pound of dried bacterial cells. When incorporated in poultry rations, the bacterial product supplies all of the vitamin B₁₂ requirement.

Research on the microbiological production of vitamin B₁₂ was also conducted by the Northern Regional Research Laboratory under an RMA project. A screening procedure, developed to survey the laboratory's culture collection for organisms capable of producing the important vitamin B₁₂, permitted the preliminary examination of several thousand organisms. Among the molds and yeasts surveyed none was found that produced this vitamin when tested with the assay organism *Lactobacillus leichmanii* ATCC 4797. A considerable number of actinomycetes (principally *Streptomyces*) synthesized varying amounts of growth factor for the test organism, although the growth factor was not always entirely vitamin B₁₂. A similar survey of a thousand cultures of bacteria also revealed a small number of promising strains.

Culture-media ingredients were investigated in the development of processes for the production of vitamin B₁₂ by some of the more promising organisms. One organism in particular, *Flavobacterium devorans* NRRL-B-54, was studied. When it was grown under ideal conditions on a medium composed of corn sugar, soybean meal, corn-steep liquor, and mineral salts, it produced significant amounts of vitamin B₁₂. Small quantities of other B-complex vitamins (riboflavin, pyridoxin, pantothenic acid, niacin, and biotin) were produced simultaneously by the culture.

Good progress is being made in the solution of the basic problem of supplying the vitamin B₁₂ requirement of poultry, swine, and other animals, and supplements of microbial origin are now available commercially. Various organisms have been discovered and developed as vitamin B₁₂ sources by different industrial companies. *Streptomyces griseus* and *S. aureofaciens*, the sources of the antibiotics streptomycin and aureomycin, produce significant amounts of vitamin B₁₂ which is recovered as a byproduct. Other undisclosed organisms are grown primarily for vitamin B₁₂ production. The vitamin B₁₂ needed for feed purposes would cost about \$30,000,000, estimated at the current price of 10 cents per milligram of vitamin B₁₂. Several companies are actively engaged in trying to produce *B. megatherium* NRRL-B-938 and *Flavobacterium devorans* NRRL-B-54 to share in this market.

Riboflavin Produced Economically by Fermentation

The production of riboflavin by the yeastlike organism, *Ashbya gossypii*, in submerged aerobic fermentation, which was developed by the Northern Regional Research Laboratory, now has been demonstrated to be practical as a commercial process. An industrial firm has obtained economical yields of the vitamin in large-scale fermentations with standard commercial equipment.

Laboratory and pilot-plant studies to improve the original process, which was discussed in the 1948 report, were continued under a Research and Marketing Act project and have been successfully completed. The laboratory experiments, which showed that riboflavin yields can be increased by addition of corn sugar to the standard medium during the fermentation process, were confirmed in the pilot

plant. Yields up to 800 micrograms per milliliter were obtained by starting with the standard medium containing 2 percent sugar and then adding 1.25 percent sugar to the fermentation 40 hours after inoculation.

Preliminary laboratory studies indicated that riboflavin yields in large-scale fermentations might be further improved by adding low concentrations of certain inorganic salts to the medium containing 2 percent sugar, $\frac{3}{4}$ percent corn-steep-liquor solids, and $\frac{3}{4}$ percent animal-stick-liquor solids. Some advantages were found in reducing the sugar in the starting medium to 1 percent and adding 1.8 percent sugar 24 hours after inoculation, although there was some sacrifice in yield. Use of the normal medium involves the possibility of a drop in pH to below 4.5 during the fermentation, with probably inhibition of riboflavin formation. When a start is made with a 1-percent sugar medium, however, the pH reaches a minimum of only 5.0, with little or no inhibition of vitamin formation. In addition, riboflavin production in the latter medium begins about 20 hours after inoculation instead of after 30 or 35 hours, the usual time when the normal medium is used.

A search was made for superior riboflavin-producing substrains or mutants that might be obtained through exposure of *Ashbya gossypii* NRRL Y-1056 to ultraviolet radiation, X-radiation, methyl-bis-B-ethylamine hydrochloride, or sodium thiosulfate. A satisfactory method was developed for laboratory evaluation of vitamin production by such substrains. By adopting a strict regimen, involving procedures for maintenance of the stock culture, preparation of the inoculum, and the use of reduced amounts of inoculum, substantial increases in yields resulted, along with greater reproducibility. Yields up to 1,760 micrograms per milliliter were obtained in the laboratory when the technique of feeding additional limited amounts of sugar to the fermenting cultures was used and when peptone replaced the animal-stick liquor. These yields compared very favorably with those produced by *Eremothecium ashbyii*, the only other micro-organisms known to give yields of such magnitude.

Toxicological and Pharmacological Tests Made on New Products

Subtilin

When the antibiotic subtilin was being investigated for its possible therapeutic value, the Pharmacology Laboratory made studies to determine the best route of administration to achieve bactericidal or bacteriostatic levels of subtilin in the blood stream. It was found that oral, subcutaneous, or intramuscular administration failed to produce satisfactory concentrations of subtilin in the blood. While these observations militated against the therapeutic use of subtilin, they suggested that the use of subtilin in food processing would not present an acute-toxicity problem for the consuming public.

There remained the problem of chronic toxicity which might arise from the eating of subtilin-containing food over long periods of time. Such chronic toxicity could be due directly to the subtilin itself or indirectly to its bactericidal action on the beneficial intestinal flora. Feeding experiments were conducted on albino rats for 200 days with diets containing as much as 0.1 percent subtilin, which is 50 times

the amount usually found effective against food-spoilage organisms. No toxic effects were noted as judged by growth, general appearance, behavior of the animals, and absence of gross abnormalities at time of autopsy.

Tomatine and tomatidine

The fungicidal properties of tomatine and tomatidine suggest therapeutic and other uses of these compounds which require a knowledge of their acute and chronic toxicities and pharmacological properties. If harmless, these substances, isolated from tomato plants by the procedures of the Biologically Active Chemical Compounds Division, might be useful for addition to foods to prevent mold growth and for treatment of fungus diseases in man and animals. Therefore, under an RMA project, studies have been made with laboratory animals on the acute, subacute, and chronic oral toxicities, on the irritant effects of surface applications, and on the cause of death after intravenous administration. The acute oral toxicity of tomatine is very low, nearly 1 gram per kilogram of body weight being required to cause death in rats.

Daily administration by stomach tube of 250 milligrams (one-fourth gram) of tomatine per kilogram of body weight produced no toxic effects. Rats fed diets containing 0.04 percent of tomatine or tomatidine for 200 days showed no gross or microscopic evidence of toxicity. Application of tomatine ointment to the skin daily for two weeks produced no irritation. Subcutaneous injections of tomatine caused ulcers or abscesses. Application of tomatine to the eye caused temporary inflammation. Intravenous injection of a few milligrams of tomatine produced hemolysis of red blood cells, a marked drop in blood pressure, and sometimes death. The fall in blood pressure was shown to be due to the action of tomatine on the central nervous system, since cutting of the vagus nerves, or atropinization of the animal, prevented the effect on blood pressure. These data indicate that tomatine is safe for surface application or oral administration, but that it should not be injected intravenously or subcutaneously.

Cottonseed pigment glands

It is rather generally recognized that improperly processed cottonseed meal produces toxic effects when used in animal feeds. It is now recognized that the toxicity is associated with the pigment glands. The investigations on the toxicity of cottonseed pigment glands by the Pharmacology Laboratory have yielded several interesting results, which point to the desirability of removing or detoxifying the pigment glands of cottonseed meal destined for use in animal feeds. Rats placed on a diet containing as much as 32 percent of cottonseed meal free of pigment glands showed no signs of toxicity. But rats receiving pigment glands in the diet showed a decrease in fertility, which was proven to be an effect on the male rats and associated with decreased spermatogenesis. The rats receiving a diet containing pigment glands ate less food than did the control rats fed a normal diet. However, inhibition of growth in the former could not be accounted for entirely by the decrease in food intake; this was proved by paired feeding experiments in which control animals had

their food intake limited to the amount consumed by the rats eating a diet containing pigment glands.

Penicillin Still Foremost Antibiotic Drug

The past decade has witnessed the development of a new, large, and expanding fermentation industry devoted to the production of antibiotic drugs from micro-organisms. The first drug of this type was penicillin, and thus far it has remained the foremost antibiotic. The successful evolution of a manufacturing process for large-scale production of penicillin was largely based upon researches conducted during World War II at the Northern Regional Research Laboratory.

Now, 5 years after these investigations were terminated, the industry is still using fermentation media that are based upon the corn steep-liquor and lactose medium developed in this laboratory. Likewise, the industry is still employing penicillin-producing molds which were derived from a superior strain that was isolated, tested, and first recommended by the same research team. During the 7-year period from March 1943 to March 1950, production of penicillin in this country climbed steadily from less than a half-billion Oxford units per month to more than 12 trillion units per month, accompanied by a decline in the wholesale price from \$20 to less than 10 cents per 100,000 units. Present production represents the output of at least 12 major drug companies.

The development of the penicillin industry has provided important new outlets for certain byproducts derived from the processing of milk and corn. The drug has saved an untold number of lives and has contributed immeasurably to the health of our people. Perhaps of even greater ultimate importance, it has opened the way to the discovery and isolation of additional antibiotic drugs of great usefulness produced by micro-organisms; for had penicillin proved less spectacularly successful, it is doubtful whether such other antibiotic drugs as streptomycin, chloromycetin, aureomycin, and terramycin would be available today.

New Streptomycin Isolated and Identified

During screening tests made recently under a Research and Marketing Act project at the Northern Regional Research Laboratory on micro-organisms of the genus *Streptomyces*, a new species was isolated which produced an antibiotic having an antibacterial spectrum similar to that of streptomycin. Careful physiological and morphological comparison with streptomycin-producing strains of *Streptomyces griseus* and *S. bikiniensis* showed that the new species, isolated from a Japanese soil, was not identical with those organisms or any other recorded *Streptomyces* species. The slow change from a grayish-white to a flesh-colored aerial mycelium exhibited by colonies of the new organism suggested the designation *Streptomyces griseo-carneus*.

Because of certain reported therapeutic shortcomings of streptomycin, it was thought desirable to investigate the antibiotic produced by the new species in the hope that it might have the valuable properties of streptomycin without some of its defects. After demonstrating conclusively, by means of paper chromatography, that the

new antibiotic was different from streptomycin and mannosidostreptomycin, the product was isolated in the pure form. Analytical data showed that the substance contains one more oxygen atom to the molecule than is present in streptomycin. Through degradation experiments, it was shown that the new streptomycin has the same streptidine and N-methyl glucosamine fragments that are present in streptomycin. Treatment of the new streptomycin with alkali, however, yielded, instead of maltol, a crystalline compound which appeared to be a monohydroxylated maltol. On the basis of this evidence, it is assumed that the new streptomycin differs in chemical structure from ordinary streptomycin only by having a hydroxyl group in place of one of the hydrogens of the streptose methyl group. Hence the new streptomycin has been given the name "hydroxystreptomycin."

Preliminary pilot-plant experiments indicated that the production of sufficient material for toxicological tests should present no great difficulty. Until the results of these tests are forthcoming, no statement can be made regarding the new antibiotic's therapeutic promise. However, because of its possible importance, every effort is being made to speed this work.

Antibiotics Isolated From Vegetables and Fruits

Diseases that are caused by gram-positive bacteria are reasonably well controlled by a number of antibiotics. However, diseases caused by gram-negative bacteria, acid-fast bacteria, fungi, amoebae, and viruses are not effectively controlled. For example, there is no known drug that will cure systemic fungus infections. However, it has been found that plant extracts contain substances which, in laboratory tests, are effective in retarding or in retarding and destroying the growth of many important disease-causing micro-organisms. Under a Research and Marketing Act project, the Bureau's Biologically Active Chemical Compounds Division has continued its research on antibiotics from agricultural sources.

Grapefruit peel

Extracts prepared from grapefruit peel and membranes, a byproduct of the grapefruit-processing industry, were found by laboratory tests to be very effective for inhibiting the growth of fungi and Mycobacteria. None of the known constituents of grapefruit peel, such as naringin, naringenin, phloroglucinol, and *p*-coumaric acid, would account for the observed antibiotic spectrum of grapefruit-peel extracts. Two crystalline antifungal agents were isolated from the extracts. One fraction, insoluble in petroleum ether, is a white microcrystalline substance having a melting point of 295°-300° C. Infrared analysis indicates that it has a sterol nucleus. The second fraction, soluble in petroleum ether, has a melting point of 128° to 139°, which indicates that it may be a mixture of two or more substances. The active principle in this second fraction may also contain a sterol nucleus.

Bananas

Bananas ripened under unfavorable conditions fail to develop antibiotic activity to the same extent as properly ripened bananas. In the case of bananas ripened at 60° F. and 70 percent relative humidity, antifungal activity appeared in the pulp and skin when the banana reached the stage represented by the color index 4 and 6, respectively, whereas antibacterial activity appeared when the color index was 4 and 5, respectively. However, the antibacterial factor that is active against *Mycobacterium phlei* did not appear in ripe bananas until a color index of 7 or 8 was reached. A commercial sample of banana powder was found to contain no factor active against *M. phlei*, thus indicating that the product was not prepared from ripe bananas having a color index of 7 or 8.

Countercurrent distribution methods were used for the fractionation of banana extracts. A complete separation of an antifungal fraction from other antibiotic substances was achieved, and work on the purification of this factor is in progress. The antibacterial factors are associated with the sugar-containing fraction.

In order to circumvent the difficulties encountered in drying the pulp and skins of ripe bananas, a new procedure was devised for the initial extraction of the antibiotic material. It is based on the observation that the aqueous extract of ripe bananas, separated by centrifugation, possesses only antibacterial activity, whereas the fibrous, solid material remaining in the centrifuge contains the antifungal principle. The antibacterial fraction has been concentrated and purified, and a very small quantity of needle-shaped crystals has been obtained. These crystals dissolve in water and in 95-percent ethanol, to yield acidic solutions, but are insoluble in chloroform and petroleum ether. Preliminary assays of an aqueous solution of the crystals showed them to be active against *S. aureus*, *E. coli*, and *M. phlei*.

Sweetpotato plant

Extracts prepared from wilt-susceptible sweetpotato stems contain antibiotic substances that are inhibitory toward gram-positive bacteria, acid-fast bacteria, and fungi. Purification of the antibiotics may be achieved by several procedures, but the best method is to absorb the inhibitory substances on a charcoal column and then elute them with a mixture of acetone and water. The elute, when concentrated, yields a lemon-colored solution that is acidic in reaction. Upon the addition of alkali, the color of the solution changes to reddish brown. This change in color appears to be characteristic of certain naturally occurring 1,4-naphthoquinones. It was possible to prepare two crystalline derivatives of the inhibitory compound. Its phenylhydrazone, a bright-yellow compound, melts with decomposition at 194°–200° C, while its 2,4-dinitrophenylhydrazone, an orange-colored compound, melts at 210°–215° C. Indications are that the inhibitory compound can also be extracted from the stems directly with sodium carbonate solution at room temperature.

Cabbage

A simplified procedure for obtaining the crude resinous substance in cabbage, which is highly active against fungi and *S. aureus*, was worked out. The compound that is responsible for the antibiotic activity of the resin appears to be different from that in the waxlike fraction, reported earlier, which is only active against fungi. Two crystalline derivatives were obtained from the resinous substance, but attempts to liberate the active component were unsuccessful. Infrared spectral data were helpful in establishing that the compound contains carbonyl and hydroxyl groups.

Summary

Plants continue to show promise as a source of new compounds having antibiotic activity. No experimental evidence has been obtained which would indicate that the antibiotics in the plants under investigation are identical. In addition to purifying and isolating the antibiotics in the sweetpotato plant, cabbage, and banana, two new crystalline compounds having antifungal activity were isolated from grapefruit peel, a byproduct of the grapefruit industry. Attempts will be made during the current year to prepare a number of these compounds in quantities sufficient for pharmacological evaluation.

Possible Medicinal Uses of Tomatine and Tomatidine Being Studied

The 1 pound of crystalline tomatine, a glycosidal alkaloid isolated from the tomato plant, which was reported as having been prepared last year, was used for the following purposes: (1) Identification, (2) determination of molecular structure, (3) toxicity studies in cooperation with the Bureau's Pharmacology Laboratory, (4) antifungal tests with animals in cooperation with Duke University School of Medicine and the National Institutes of Health, (5) antimalarial and amoebicide tests with animals in cooperation with the National Institutes of Health, and (6) synthesis of sterols in cooperation with the National Institutes of Health.

Tomatine and tomatidine were found to be essentially nontoxic when fed to experimental animals in chronic and subacute toxicity tests, and when applied externally. In view of the favorable results of oral toxicity tests, determination of the structures of these compounds was of considerable importance. Tomatine yielded one molecule each of tomatidine, xylose, and galactose, and two molecules of glucose when hydrolyzed with acid. Tomatine and tomatidine gave discrete X-ray powder patterns.

The pattern of a humidified (wet) tomatidine sample differed from that of a dry sample, thus indicating hydrate formation. Tomatidine was found to contain no chromaphoric groups possessing characteristic absorption in the ultraviolet region; therefore conjugated unsaturation of both the aliphatic and aromatic types is missing. Likewise, uncombined keto or aldehydic groups are absent. In optical comparisons with related sterol compounds, the infrared data showed clearly that tomatidine contains one hydroxyl group and one secondary amino group. Since tomatidine contains only one hydroxyl group and no carbonyl group, it was evident that the second oxygen must

be in an oxidic linkage. No methoxyl group was found; therefore, the second oxygen indicates a ring ether. Empirical formulas have been assigned to tomatidine ($C_{27}H_{45}O_2N$), N,O-diacetyl tomatidine ($C_{31}H_{49}O_4N$), and tomatine ($C_{50}H_{83}O_{21}N$). Also a structural formula has been proposed for tomatidine. This compound appears to be related chemically to the veratrum alkaloids, and its cardiac activity is therefore worth investigating.

The possible use of tomatine and tomatidine in the treatment of systemic fungus infections, amoeba infections, malaria, and other diseases has been considered. It is still unknown whether tomatine and tomatidine are absorbed through the intestines, but since they were found to be nontoxic when administered orally, samples of these compounds were supplied to the National Institutes of Health and to Duke University School of Medicine for animal experimentation with diseases involving amoeba, malaria, and systemic fungus infections. Only preliminary results are available at this time.

Some consideration was also given to the possibility that tomatidine might be a starting material for the synthesis of therapeutic sterols. After consultation with scientists in the National Institutes of Health, a 50-gram sample of tomatine was forwarded to them for such investigations.

Plant-Growth Regulators Found in Various Plant and Animal Products

Under a Research and Marketing Act project, the Biologically Active Chemical Compounds Division has continued its search for sources of natural plant-growth regulators. Particular emphasis was placed upon certain commodities on which the Bureau is now working. Several green plant materials supplied by the Regional Research Laboratories were found to be active according to the standard assay procedure. The most promising sources of natural plant-growth regulators were dried beet tops, lima-bean meal, turnip leaves, rhubarb, spinach, broccoli, cabbage, and corn. In addition, such widely different materials as corn-steep solids, animal-stick liquor, and spray-dried soybean-whey solids were found to possess plant-growth regulating activity.

Last year's report stated that two plant-growth regulators are present in distillers' solubles, a byproduct in the production of alcohol. By means of the automatic multiple extraction apparatus, developed by this division, one of these plant-growth regulators was purified to such an extent that it was active at 1-to-50,000 dilution. However, the other plant-growth regulator (the toxic factor) did not yield to purification and seemed to be nonspecific in its action. It is of interest to note that the ultraviolet-absorption spectrum of a chick-growth-factor concentrate, called vitamin B_{13} , coincides with that of the division's most purified fraction of plant-growth regulator. This observation has been reported, and commercial companies interested in the vitamin B_{13} content of distillers' solubles are now investigating the possibility that this plant-growth regulator is the same as the chick-growth factor.

One of the active plant-growth regulators in corn pollen produces cell elongation. The most active fraction obtained thus far was

active at 1-to-1,000 dilution, which represents a hundred-fold increase in specific activity over the original material. Ultraviolet spectrophotometric studies on this fraction showed a strong absorption band at 280 millimicrons. Studies on the completeness of extraction of plant growth regulating activity from corn pollen confirmed earlier observations on the difficulties involved in extraction. Ethyl acetate and moist ether were both good solvents, but, even after successive extractions of corn pollen with these two solvents, dilute sodium bicarbonate solution removed more activity from the residue. On the other hand, when aqueous sodium bicarbonate was used as the first solvent, considerable residual activity was extracted by moist ether. A further purification of this fraction will be necessary before identification becomes possible.

New Type of Plant-Growth Regulator Tested and Prepared in Quantity

A new type of highly active plant-growth regulator has been discovered. Two new compounds, selected from a list published by the Chemical and Biological Coordination Center of the National Research Council, as being available to research workers, and procured through that organization, were tested for their effects on plant growth. They were carbamic acid esters of 4-dimethylamino thymol and 4-dimethylamino carvacrol methiodides.

Both of these compounds, when applied to bean plants, greatly reduced the rate of growth and modified the structure of plants grown under adverse lighting conditions and in poor soil, as compared with control plants grown under the same conditions. The effects were more pronounced with one compound than with the other. The control plants were tall and spindly; their leaves were light green in color; and their seed pods were small and immature at the end of the testing period. In contrast to the controls, the treated plants were short and stocky (about one-third as high); the leaves were dark green in color and rippled (but normal in size); and the seed pods were normal in size and appearance and were present in greater numbers. The most striking difference between the controls and the treated plants was that the latter had very short internodes, which gave the plants a very bushy appearance because of the tight corolla-like formation of each successive group of leaves.

Since more of these compounds was not available from the original source, they have been synthesized in sufficient amounts by the Biologically Active Chemical Compounds Division for large-scale testing by the Bureau of Plant Industry, Soils, and Agricultural Engineering. New analogues and homologues are now being synthesized.

A sample of the new compound that showed the greater effect on plant growth was supplied on request to the Sloan-Kettering Institute for Cancer Research, at Memorial Hospital, New York City, for testing as a cancer-suppressing agent. A number of other plant-growth regulators, namely, 2,4-dichloro-5-iodophenoxyacetic acid and its derivatives, are also being tested there for the same purpose.

Plant-Growth Regulators Act on Enzyme Systems

It appeared probable that plant-growth regulators entering a plant may combine with one or more of the essential nutrients of the plant or in some manner catalyze or inhibit certain enzyme systems. Amino acid and sugar derivatives of plant-growth regulators appeared to be a likely formation, since, generally in plants, the starch reserve is depleted, and the nitrogen reserve is increased, under the influence of some regulators.

The syntheses of N-2,4-dichlorophenoxyacetyl and N-2,4,5-trichlorophenoxyacetyl derivatives of alanine, leucine, lysine, methionine, aspartic acid, and glutamic acid have been accomplished. When tested for plant growth regulating activity in the lanolin-bean test by the Bureau of Plant Industry, Soils, and Agricultural Engineering, these amino acid derivatives were found to be as active as 2,4-dichlorophenoxyacetic acid (2,4-D) itself. Thus, in chemical combination with amino acids, the activity of the plant-growth regulator is not diminished. It is postulated that amino acid derivatives may increase the rate of absorption and distribution due to the particular polar or surface-active groups present.

2,4-Dichlorophenoxyacetyl derivatives of glucose were also prepared and tested. They were found to be active plant-growth regulators. As was the case with the amino acid derivatives, no inactivation was observed.

These results indicate that 2,4-dichlorophenoxyacetic acid (2,4-D) does not inhibit plant growth by combining with amino acids or simple sugars through a reaction involving its carboxyl group. Indeed, when an 80-percent ethanolic extract of dried tops from young bean plants treated with radioactive morpholine 2,4-dichloro-5-iodo¹³¹-phenoxyacetate was chromatographed, the radioactive material was found to move much faster than did the sugar. This would indicate that the regulator was not attached to this plant constituent.

To obtain more conclusive evidence, the radioactive portion on the paper chromatogram was eluted and hydrolyzed with acid, then neutralized, and again chromatographed. It was found that the radioactive material located in the same position on the paper chromatogram as before. There was no detectable amount of sugar present. Therefore the regulating activity must be due to catalyzing or inhibiting some enzyme systems in the plant rather than to combination with carbohydrate compounds.

Chemical and Immunological Studies Continued on Allergens

Under an allotment from the special research fund authorized by the Bankhead-Jones Act of June 29, 1935, the Bureau continued researches on allergens of agricultural products. The object of these studies is to find which of the many complex organic components of agricultural products are capable of inducing allergic diseases.

Isolation and immunological characterization of allergens acquires increasing significance as supporting evidence in collateral fields of clinical research. The dramatic effect of the adrenal and pituitary hormones, cortisone and ACTH, on rheumatoid arthritis has directed attention to allergic diseases which also respond favorably to the same

treatment. Certain symptomatic features are common to both rheumatoid arthritis and allergic diseases. Accordingly a common underlying biological mechanism may be inferred from the spectacular remission of symptoms of severe allergic asthma and rheumatoid arthritis following administration of cortisone or ACTH.

Cortisone, the specific, beneficial component of the adrenal extract has been isolated, and its chemical structure has been determined. In complexity of chemical composition the pituitary hormone ACTH resembles the allergens that have been isolated from the oilseeds under this project. Specificity of physiologic activity is a common characteristic both of the allergens that induce symptoms of allergic disease and of the hormones that abolish those symptoms. Thus, in those findings, a promising new approach to research on the mechanism of allergic reactions becomes apparent.

Isolation of the allergens provides materials that are essential to correlation of chemical structure and allergenic activity. This research parallels the current intensive research on the adrenal and pituitary hormones in other laboratories. One objective is common to both lines of investigation, the precise correlation of chemical composition and physiologic activity.

Oilseed allergens assayed for amino acid contents

The determination of the chemical composition of allergens by microbiological assays of their amino acid contents was continued. To evaluate their reliability, the methods were used for determining amino acids in two well known proteins of milk, namely, casein and beta-lactoglobulin. These proteins were chosen because both are obtainable in high purity and the proportions of their amino acid components have been established by previous studies of several laboratories. The values obtained in this study agreed satisfactorily with those reported previously. Determination of 18 amino acids by microbiological assays accounted for 96.5 percent of the total nitrogen of casein and 95.9 percent of the total nitrogen of crystalline beta-lactoglobulin. Thus the results of these assays supported the reliability of the microbiological methods, which have substantial advantage in economy when compared with chemical methods of protein analysis.

These microbiological methods were then used to determine the amino acid contents of the allergenic fraction CS-1A from cottonseed, and of three previously described fractions obtained from CS-1A by electrophoresis. These electrophoretic fractions, CS-54R, CS-55R and CS-56R, and the CS-1A from which they were derived were prepared from depigmented, defatted cottonseed, a product of another phase of the Bureau's research on cottonseed. The electrophoretic fractions represented purification of the allergenic fraction CS-1A, with accompanying decrease in the proportion of uncombined and chemically combined carbohydrate. In general the relative proportions of the amino acids in CS-1A paralleled those of the more highly purified fractions in which the carbohydrate content was considerably less. A previously noted characteristic—the exceptionally high proportions of the amino acids arginine and glutamic acid—was evident in all these fractions.

The continued attempts to purify the allergenic fractions of oilseeds and to improve the precision of their assay for amino acids were de

signed to reveal either significant similarities or differences that may be related to the capacity to induce allergic reactions. Therefore, for comparisons, the amino acid contents of allergenic fractions of soybeans, peanuts, and short rag-weed pollen were determined.

With respect to allergenic properties, as manifested by reactions of experimental animals, the soybean and peanut allergens are not typical of the allergens represented by the 1A fractions of other oilseeds, of which cottonseed and castor bean are thus far the most critically studied examples. Possibly this notable difference in allergenic activity of similarly isolated fractions from similar oilseeds signifies partial destruction or alteration of structure of some component during the fractionation process. Evidence favoring this hypothesis, and exemplifying the value of amino acid analyses, was derived from the findings that the fractions soybean 1B, peanut 1B, and short rag-weed pollen 1A contained significantly lower proportions of arginine and glutamic acid than were found in the cottonseed fraction CS-1A and in the electrophoretic derivatives of CS-1A. On the other hand, the proportions of lysine in the allergenic fractions soybean 1B, peanut 1B, and short rag-weed pollen 1A exceeded by several times the proportion of this amino acid in cottonseed 1A. Glycine accounted for about 30 percent of the total nitrogen in the peanut 1B allergen—a much higher proportion of this amino acid than was found in any other allergen. Such differences in composition can be said to correspond with significant differences in immunologic behavior of these allergens, but the biologic mechanism of the allergic response is not yet explainable on the basis of present information gained from chemical studies.

Another interesting comparison concerning amino acid content and biologic activity resulted from microbiological analysis of protein fractions prepared by the Allergen Research Division from culture filtrates of human and bovine tuberculosis bacteria. These allergenic preparations were higher in arginine than were soybean and peanut allergens but lower in this amino acid than the typical oilseed 1A fractions. Tuberculin proteins contained a higher proportion of the amino acid tryptophan than any of the oilseed allergens except the 1A fraction from flaxseed. Allergenic activity of the oilseed protein has been shown by previous studies under this project to be independent of tryptophan content.

Research findings fortify clinical evidence of castor-bean allergy

Recent observations of allergists have renewed public interest in the prevalence of castor-bean allergy among people who have no obvious contact with castor beans or castor pomace. Clinical studies have shown that the castor-bean allergen is unique in its capacity to establish sensitiveness, with resultant symptoms of allergy, by comparatively brief and slight exposure to dust from the castor seeds or castor pomace, the residue of milling for recovery of castor oil.

Castor-bean milling practices and a prospective need to develop domestic sources of castor oil for expanding industrial purposes directed attention of this Bureau to potential hazards arising from castor-bean allergen as a contaminant of edible and other commodities. Complete avoidance of sources of castor-bean allergen would appear to be assured among all but those engaged in production, transportation,

or processing of the seed or pomace. However collaboration with allergists practicing in industrial centers has recently produced evidence which implies that exposures to castor-bean allergen may be more common and more serious, from the viewpoint of public health, than has been suspected from hitherto infrequent reports of castor-bean allergy.

Within the past year this Bureau was notified of allergic asthma among employees of a coffee-roasting plant. Exposure to dust from green coffee provoked symptoms of allergy which appeared to be associated with a coincident sensitiveness to castor-bean allergen. Clinical evidence based on differentiating tests with green-coffee dust and with the castor-bean fraction, CB-1A, supplied by the Allergen Research Division, implied that the green coffee had been contaminated with castor bean or castor pomace. Techniques developed in the Bureau's research on oilseed allergens were applied to determine whether green coffee contained a coffee allergen that might affect those who are sensitive to castor bean.

Immunologic examination of the suspected coffee dust proved the presence of castor-bean allergen and provided conclusive evidence that serious allergic effects resulted from exposure to green-coffee dust containing the castor-bean allergen as a contaminant in the proportion of one part per million. Additional samples of green-coffee dust and other coffee products are being examined to determine whether contamination with castor-bean byproducts is exceptional. Data available from the present study support the allergists' conclusion that asthma and related allergic symptoms attributed to breathing dust from green coffee are solely manifestations of castor-bean allergy.

Both unmilled castor beans and castor pomace contain, in addition to the allergenic protein, an exceedingly toxic protein called ricin. Danger of poisoning from ricin has prevented use of pomace for livestock feeds, even though the ricin can be detoxified by suitable heat treatment. Accordingly the principal economic value of pomace depends on its virtues as an organic source of nitrogen and other plant nutrients in commercial fertilizers. Allergic asthma caused by castor pomace in fertilizers has been recognized by allergists, and asthma among freight handlers has been noted and associated with dust from commodities shipped in burlap bags. Castor-beans and castor pomace, green coffee, grains, and numerous other commodities are transported in burlap bags or wrappings. The open weave of burlap and reuse of burlap bags may account for the presence of the castor-bean allergen as a contaminant in green coffee and in the fibers of used burlap. Identification of castor-bean allergen as a contaminant implies, also, the presence of other components of castor-bean which would include at least traces of the toxic protein ricin. The significance of ricin in less than obviously harmful amounts has not been determined, but the extraordinary potency of castor-bean allergen may account for previously unexplained occurrence of allergy from dusts of unidentified composition.

Immunologic methods identify milk proteins

A study of the immunological relationship of proteins in cow's milk to the proteins in blood serum was undertaken by the Allergen Re-

search Division in collaboration with the Protein Division of the Eastern Regional Laboratory.

For many years the relationship between blood serum and milk proteins has been the subject of repeated study with variable and contradictory results. There is general agreement that colostrum and normal milk contain protein that is serologically identical with immune globulin in blood serum. Colostrum is particularly rich in this immune globulin. The fraction of milk commonly called lactalbumin had been shown to consist largely of a readily crystallizable globulin—beta-lactoglobulin—and it was questionable whether milk contained a true albumin. This question has now been answered.

The Eastern Regional Research Laboratory obtained evidence that a small component of whey proteins has the electrophoretic mobility of blood-serum albumin. This component, which was isolated in the crystalline state, appeared to be crystalline milk albumin, but it exhibited physical and chemical properties attributable to blood-serum albumin. The crystalline albumin was submitted to the Allergen Research Division for final proof of identity.

The immunological relationship of milk albumin and serum albumin was determined by the capacity of each of the two preparations to induce anaphylactic reactions and specific desensitization to the other in tissues from animals sensitized with milk albumin, serum albumin, whole blood serum and raw milk. Crystalline beta-lactoglobulin was also included in these studies because of its relationship to the so-called lactalbumin fraction of milk.

The crystalline milk albumin was shown to be immunologically identical with blood serum albumin. At least one other protein of milk, which is present also in bovine serum, was detected but not identified. It was doubtless the immune globulin identified by previous investigators.

Two distinct contaminants were detected in the crystalline milk albumin. One was identified as beta-lactoglobulin, which was present in a concentration of about 0.01 to 0.02 percent. The second contaminant was identical with some component of blood serum and, therefore, was probably immune globulin.

The beta-lactoglobulin preparation did not contain a detectable trace of albumin and, in agreement with the findings of previous investigators, was clearly distinct from any component of blood serum.

RESEARCH APPARATUS AND METHODS

Automatic Multiple-Extraction Apparatus Devised

In connection with a Research and Marketing Act project on the isolation, identification, and production of natural plant-growth regulators from agricultural sources, it became necessary to concentrate the biologically active substances in corn pollen and distillers' solubles. Recent work on countercurrent distribution had emphasized its usefulness in biochemical fractionations, but the available equipment was not adequate for the scale of work required. Therefore an apparatus was devised in which large volumes of solvent could be handled with little effort. In principle, it differs somewhat from the

Craig apparatus, but the end result, namely, the separation of solutes having different distribution coefficients, is similar. By the use of this apparatus, it has been possible to concentrate the active plant-growth regulator in distillers' solubles from a crude preparation active at 1-to-100 dilution to a fraction active at 1-to-50,000 dilution.

The apparatus consists of a series of side-arm test tubes, which may be mounted on a vertical stand, each containing an inner tube having a small funnel at the top and a fritted disc at the bottom. The lower layer of an equilibrated solvent pair is placed in each of the test tubes, and the upper layer is placed in a reservoir mounted on a stand above the highest tube. The test tubes are so arranged that the overflow from each runs into the funnel top of the inner tube of the next lower one. The lighter member of a solvent pair is introduced continuously and at a properly regulated rate into the first dispersion tube. Drop-lets of upper phase enter the lower phase through the fritted disc, rise to the top, and overflow into the next dispersion tube. The extractions thus take place almost automatically, and, once a suitable flow rate has been established, very little further attention need be given until the process is completed. The number of tubes necessary for a particular purification, as well as the total volume of upper phase required for any degree of separation of two or more solutes, can be predicted from their respective distribution coefficients.

The mathematical analysis of data obtained with the Craig apparatus is based upon the binomial expansion formula, whereas, with the apparatus developed by the Bureau's Biologically Active Chemical Compounds Division, the expression for the concentration of a given fraction is derived from a series of differential equations based on the assumption that all of the solute was originally in the first tube, and that complete equilibration is reached during the passage of each small droplet of upper layer through the lower layer. Thus, the experimental data obtained with this multiple-distribution apparatus have shown that the distribution follows the theoretical pattern of a Poisson exponential distribution. Its effectiveness in the separation of compounds having slightly different distribution coefficients has been verified. Because of the widespread use of Poisson distribution, compilations of the individual terms, as well as accumulation of terms, have been published, and all of the data necessary for drawing theoretical curves and predicting the necessary conditions for the separation of two or more solutes are available in tabular form. This apparatus will undoubtedly find many uses in the preparation and purification of biologically active compounds.

Special Paper-Partition Chromatographic Apparatus Developed

The development and application of paper chromatographic techniques to chemical problems has been one of the outstanding advances in recent years. As a result, many important problems have been solved, and others are being solved. The Bureau's research on antibiotics from agricultural sources, conducted under a Research and Marketing Act project by the Biologically Active Chemical Compounds Division, has been speeded up by the development of a special chromatographic apparatus. It has the advantages of simplicity, convenience, flexibility, minimum expense for material, small space

requirement, and an air-tight system. The apparatus consists of a 1- or 2-liter graduated cylinder, a stainless-steel paper holder, a rubber stopper, and large sheets of filter paper. The cylinder can easily be protected from light, if the compounds are light-sensitive, and the paper holder can be made of glass, if conditions demand it. By the use of multiple units of this apparatus, it has been possible to detect the different antibiotics in plant extracts. In other instances, the identification and quantitative separation of sugars have been accomplished, and important advances have been made in the investigation of the action mechanism of plant-growth regulators. A description of the apparatus was published in the scientific journal *Science*, volume 110, pages 232-233 (1949).

Addition of Triethylamine Increases Applicability of Infrared-Transmitting Solvents

Infrared analyses are providing useful "fingerprints" of organic compounds for such important fields of research as cancer, arthritis, antibiotics, and others related to medicine. When infrared spectra of solids in the solution state are desired, it is frequently impossible to find a suitable solvent. Carbon disulfide and carbon tetrachloride are outstanding examples of the type of solvents that have sufficient infrared transmittancy for use over extensive portions of the solvent region, but many of the organic substances under investigation will not dissolve to the necessary extent in these liquids. Some acids, for which no other suitably transmitting solvent could be found, readily dissolved in modified solvent mixtures developed by the Biologically Active Chemical Compounds Division.

The two solvents considered to be the best for infrared investigation, namely, carbon disulfide and carbon tetrachloride, were modified by the addition of 0.5 and 2.5 percent of triethylamine. The resulting mixtures transmit sufficiently well for easy use in all of the regions from 2 to 15 microns, excepting those already obliterated by the unmodified solvents. With the mixed solvent in the balancing cell, the corrected curve obtained is neither that of the free acid nor that of the triethylamine salt, but approaches a representation of the neutralized acid without the pattern of the neutralizing base. The curves obtained are characteristic of the compound and are generally useful in the ways of normal infrared spectra, but close comparison of two compounds for identity and quantitative effects are best made with data obtained on each in the same manner. Band locations are generally unchanged in position, with few added or subtracted peaks. There are, however, characteristic differences, some of which can give information of importance to supplement that obtained from the spectra of the free acids.

These mixtures have been of considerable help in obtaining characteristic patterns for plant-growth regulators, such as 2,4-dichlorophenoxyacetic acid (2,4-D) and related acids for which no other suitable infrared-transmitting solvent could be found. Analogous solvation methods might possibly be applied for extending the solubility of basic substances in carbon disulfide and carbon tetrachloride, and thus a wider application of this technique could be developed.

Improved Analytical Methods Developed for Cottonseed Products

Cottonseed meal and oil, like many other items of commerce, are bought and sold on the results of analysis by specified methods. Hence, better methods are sought constantly as a means of establishing equity between buyer and seller and to facilitate business transactions in the marketing of cottonseed and cottonseed products. Better analytical methods also speed research.

During the year two additional analytical methods were developed by the Southern Regional Research Laboratory incidental to its research on the processing of cottonseed and on the nutritive value of cottonseed cake and meals. They are methods for the determination of total gossypol pigments in cottonseed cake and meals and in cottonseed oils. When used in conjunction with the method previously reported for the determination of free gossypol pigments in cottonseed materials, these new methods provide a means of accounting completely for the gossypol pigments during seed processing.

In order to determine the total gossypol pigments in cottonseed cake and meal, the bound pigments are liberated by hydrolysis with a solution of oxalic acid in aqueous methylethyl ketone by heating at 75° C. for 16 hours or overnight. For their determination in cottonseed oils, the oil is dissolved in a mixture of commercial hexane and isopropanol. In both cases the total gossypol pigments are finally evaluated colorimetrically after reaction with *p*-anisidine as specified in the method for free gossypol pigments, previously reported. These methods are being used in the research of the Southern Laboratory and have been made available to industry.

Method Found for Locating Double Bond in Oleic Acid Isomers

Vaccenic acid, an oleic acid isomer having the double bond between carbons 11 and 12, is said to be responsible for the increased nutritive value of summer butter.

In attempts to synthesize vaccenic acid, the problem of identifying it among other oleic acid isomers was encountered. At the Northern Regional Research Laboratory a solution of this problem was found by X-ray diffraction after converting this unsaturated acid to dihydroxy stearic acid. The X-ray diffraction patterns of the derivative showed that its hydroxyl groups were in the 11-12 positions and that the parent compound was therefore vaccenic acid. Comparison with the pattern of a dihydroxy stearic acid derived from elaidic acid, having the double bond between carbons 9 and 10, showed marked intensity differences, thereby providing a rapid method of identifying these two position isomers.

Measurement of Food-Quality Differences by Taste Panels Made More Accurate

It is well recognized that those differences in quality of foods that can be detected by tasting, play a large part in determining which food the consumer will buy. Therefore, the changes that occur during processing, storage, and distribution of foods must be measured by taste tests or by some other test that has been found to correlate with the results of taste tests. Because highly important

decisions, in industry as well as in research, frequently rest on the data obtained from taste-panel results, it is essential that food scientists make every improvement possible in this method of testing.

It is characteristic of taste tests that a group or panel of persons frequently gives the same food samples widely different flavor scores at different times. Interpretation of results must, therefore, be made with extreme care. Also where small differences are of interest, as in the study of the initial stages of flavor changes in food, the judges (panel members) must be selected for acuity, and their interest in accurate tasting must be maintained. Finally, the scores assigned to certain samples can be reliably compared with scores assigned to other samples judged at the same time but not with the scores of samples judged at some other time. This last difficulty, combined with the finding that only a limited number of samples can be reliably scored by a judge at one sitting, has led to the use of a new statistical design for presenting samples to judges.

This statistical design, which has been used in other fields, is known as the balanced incomplete block design. It is especially useful where comparisons are needed for more than four samples, which generally is the maximum number of samples that it is desirable to present to a panel of judges at one sitting. In a case where it was necessary to compare 10 samples of dried eggs, the samples were grouped in 15 blocks or sets of 4 samples each in accordance with a balanced incomplete block design. The samples were so grouped that each sample was tasted six times and each was compared directly with every other sample twice. In this way a statistical comparison of all samples was obtained, even though it was not satisfactory to taste all 10 samples at one sitting. The design has been used successfully at the Western Regional Laboratory for comparing 5 to 10 samples in 15 experiments with poultry, eggs, and vegetables. Use of the design does not eliminate the need for selecting and training the tasters, or for continually rechecking their performance to be sure that their acuity or interest has not decreased.

The design has advantages in that it saves the time of judges, saves material, helps to maintain the interest of the judges, and provides reliable quality comparisons among as many as 10 to 12 samples of food.

Studies in the taste-testing field have been carried along at the same time as chemical and technological studies of foods, and many comparisons have been made of the results of tasting with the results of chemical tests. The reliability of the results of tasting is supported by the consistency of the correlation of the tasting scores with results of chemical tests and other objective tests in cases where the chemistry of the deterioration has been fairly well established.

A research taste panel was established at the Northern Regional Laboratory several years ago, in connection with its work on soybean products, and is functioning with increased success. Improvements in mathematical analysis of the tasting results, better conditions of tasting, and closer control of all variable factors have improved the precision and reproducibility of the panel's work. During the past year, a conference with leaders of taste panels employed by industries showed that a majority of those testing soybean oil were modeled after this Laboratory's panel. Standard samples of soybean oil are circulated among these groups to increase uniformity of judgments.

