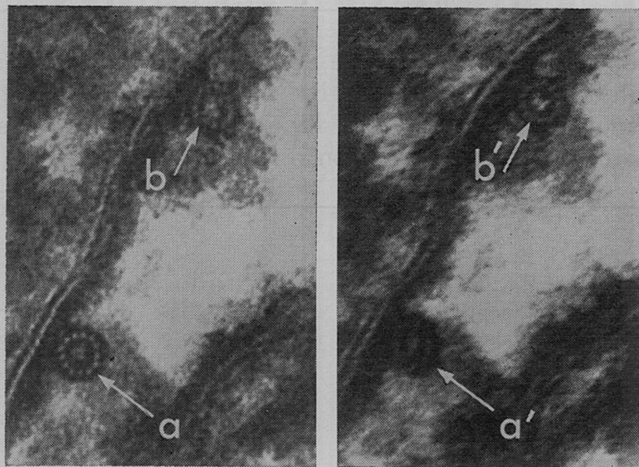

Figure 6. Microtubules from *Juniperus* seen from aspects which differ by 10°

Clear image, *a*, blurred when tilted, *a'*; blurred image, *b*, becomes clear when tilted, *b'*. ×220,000

cellulose microfibrils may be complete prior to the observed longitudinally oriented cyclosis. The mechanism by which cytoplasmic streaming or the presence of microtubules might orient the microfibrils of cellulose is difficult to fathom, especially since the locus of cellulose synthesis is unknown. In this connection, the intimate association of the microtubules with the membranes is of interest. Information may be transmitted in some way by the plasma membrane to the growing cell wall.

In their general morphology and details of fine structure the microtubules in cells of higher plants are similar to units found widely distributed in plants and animals (*2, 4, 5, 8–11, 13, 16*). It is evident from the results reported here that the wall of the tubule as viewed in transverse section is composed of 13 units and that the axis of the tubule must coincide with that of the electron beam of the microscope to within a very few degrees if the subunits are to be resolved. Such structure has been resolved in a section with an estimated thickness of 1500 A. This suggests that the wall of the tubular element is composed of an array of filaments parallel to the axis of the tubule and that these filaments must follow lines parallel to the axis to within a few angstroms for at least 1500 A. This concept compares favorably with the negatively stained images of whole tubules from the 9 + 2 complex of sperm tails (*1, 12*). The role of these tubular elements found widely distributed among plant and animal cells is probably one of providing a cytoskeletal structure which is associated with cytoplasmic movement and cell asymmetries (*2, 13*).

There is ample reason to believe that the cytoplasm controls the specific patterns of cellulose microfibrillar orientation found in the cell walls of higher plants. It is reasonable then to expect to find in the cytoplasm some anisotropic mechanism for this control. The system of cortical microtubules described here fulfills this expectation far better than any other structure of the cell cortex known to us.

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WORLD-WIDE RESEARCH

Modification of Wheat Protein for Preparation of Milk-Like Products

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Many underdeveloped countries have an insufficient amount of protein food, including low-cost protein-rich beverages suitable for small children. The present work was undertaken to explore the possibility of converting wheat protein into a dry material that would reconstitute with water to give a milk-like product. The product should be bland in flavor, milk-like in appearance (white), and have a protein solid ratio similar to milk. Lysine addition to such a material would be desirable to provide a somewhat better amino acid balance than is found in wheat. Such a material might be employed in combination with other high quality protein sources such as nonfat dry milk or soybean protein. Processing costs may limit the general applicability of methods of modifying wheat protein.

Problems in producing a milk-like

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Pepsin-HCl treatment increased the solubility and destroyed the dough-forming properties of wheat gluten. Protein was separated from wheat flour by pepsin-HCl treatment. Part of the starch fraction was modified with alpha-amylase for incorporation into the product. A dry milk-like product was prepared from wheat on a laboratory scale.

product from wheat arise because gluten proteins are water insoluble and have dough-forming elastic properties. A second aspect of the problem with gluten proteins is their isoelectric point, which is at about pH 6.6. This pH is close to that of milk and also to the value desired in the beverage. Gluten is insoluble, in part, because of the lack of sufficient charge on the protein (*3*). Whatever process is used to modify wheat proteins to obtain dispersibility and stability, it must not cause production of undesirable flavor, color, or odor, or affect the nutritional value. Browning is considered to be the most important storage problem. This means that the process used should produce a minimum of free-amino groups when the protein is modified, and a minimum of free-reducing groups when the starch is modified. A practical starting material could be flour, wheat, high-protein flour, or gluten. Some known methods for modifying flour proteins include acid hydrolysis, deamidation, hydrolysis with proteolytic enzymes, and splitting of disulfide bonds.

Hydrolysis with acids to the stage of free-amino acids, with the production of large amounts of small peptides, is unsatisfactory because of the development of a large free-amino group content.

Deamidation, or removal of the amide group of glutamine and asparagine, leaving carbonyl groups, was very effective in modifying the protein with respect to the physical properties desired. However, even a very mild deamidation produces a bitter, cardboard-like flavor. Hydrolysis with proteolytic enzymes was investigated and pepsin was the only promising enzyme for this purpose. Papain, for example, produces too many small peptides.

Pepsin treatment produces large fragments and thus relatively small amounts of free-amino groups. It also increases the acid solubility and makes possible extraction of about 85% of the protein from wheat flour by very simple techniques. Pepsin-acid treatment rapidly destroys the dough-forming and elastic properties of gluten. Splitting disulfide bonds invariably produced undesirable flavors which are unsuitable in a food product.

Since glutenin is a major component of the gluten proteins and is the least soluble of these, it was employed as a model for the study of pepsin action (*5*). The glutenin was purified by extracting vital gluten with acetic acid and precipitating with sodium acetate, using the method of Jones, Taylor, and Senti (*4*).

In general, pepsin hydrolysis of purified glutenin was carried out by using 4.47 mg. of glutenin per ml. and 47.6 μg. of pepsin in a pH 2.0 HCl solution at 40° C. Figure 1 summarizes work presented in detail elsewhere (*5*) and indicates a marked difference between rates of change of different properties of glutenin during pepsin treatment. Changes are expressed in terms of percentage of the reaction obtained during hydrolysis for 48 hours. Decrease of viscosity is the most rapid change observed. The more rapid production of pH 6.5 buffer-soluble nitrogen as compared with trichloroacetic acid (TCA)–soluble nitrogen indicates more rapid production of large peptides as compared with small ones. The patterns of these changes are favorable from the standpoint of the desired product. The disappearance of the dough-forming properties of glutenin closely parallels the viscosity decrease.

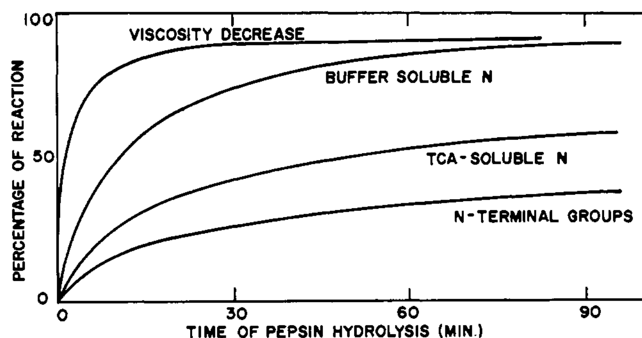

Figure 1. Comparison of changes in viscosity, soluble nitrogen, TCA-soluble nitrogen, and *N*-terminal groups during pepsin hydrolysis of glutenin

When pepsin treatment was carried out for 48 hours, 6% of the glutenin nitrogen was in the nondispersible or insoluble fraction, 78.5% was in the water-soluble nondialyzable fraction, and 15% was in the water-soluble dialyzable fraction. The action of papain on glutenin contrasts markedly with that of pepsin. When glutenin was treated with papain under conditions designed to achieve hydrolysis of essentially all of the labile peptide bonds, 15% of the glutenin nitrogen remained insoluble, 8% was in the soluble nondialyzable or intermediate molecular size fraction, and 77% was in the water-soluble dialyzable fraction.

Changes in the molecular weights of the soluble portion of pepsin-treated glutenin were observed after various times of treatment using gel filtration on Sephadex G-100. Even after 48-hour treatment, a major part of the glutenin protein still had a molecular weight in the range of 10,000 to 20,000 (*5*). To find out what specific amino acid groupings in glutenin are susceptible to pepsin action, *N*-terminal groups produced during pepsin action were studied using the method of Sanger (*6*). The content of free *N*-terminal groups of glutamic acid, valine, and isoleucine increased markedly during pepsin treatment. Semiquantitative results on *C*-terminal groups, using the method of Akabori (*1*), indicated a marked increase in leucine, phenylalanine, and alanine during pepsin hydrolysis. Since the amino acid sequence in glutenin is not known, it is concluded that some combination of glutamic acid, valine, and isoleucine as *N*-terminal, and leucine, phenylalanine, and alanine as *C*-terminal are the groupings in glutenin most susceptible to pepsin action (*5*).

In addition to modification of proteins, the starch must be modified to obtain a material that is liquid at a solids content of 13%. Treatment with alpha-amylase is suitable for modifying gelatinized starch to a product having the desired milk-like consistency at the desired solids content of 13% (*2*).

In addition to modification of protein and starch, it was also found desirable to add a stabilizer, as not all of the protein is soluble at the desired pH of 6.6. Approximately 25 different gums were

compared for achieving stabilization. Polysaccharide gum B-1459 (Commercial Solvents Corp.), a material produced by bacterial synthesis, is the most satisfactory gum found so far for obtaining good stability of the product after mixing with water. It has not yet received Food and Drug Administration approval. Various carboxymethylcellulose gums—CMC-7HT, for example—are also suitable, but they are less effective than polysaccharide gum B-1459 (*2*).

The over-all process of producing a milk-like product from wheat on a laboratory scale is given in Figures 2 and 3. Figure 2 shows the separation of protein. Hydrochloric acid 0.045*N* (3960 ml.), flour (2000 grams), and 40 ml. of a 0.1% solution of crystalline pepsin (Mann Research Laboratories) are mixed. The mixture is held at room temperature for 2 to 4 hours and centrifuged.

The supernatant contains most of the protein. The starch containing precipitate is washed with 0.001*N* HCl to remove some of the remaining protein and is centrifuged again. The original supernatant and washings are combined. About 30% of the centrifuged starch fraction will go back to the product after alpha-amylase modification. About 70% could go into the manufacture of starch. Typical yields of protein in this extraction are about 85 to 90%.

Treatment of the starch fraction comprises addition of alpha-amylase, heating at 70° C. for 30 minutes, and finally heating to 90° C. for another 30 minutes to destroy the enzyme. The neutralized pepsin–hydrochloric acid extract of wheat flour is mixed with the alpha-amylase–treated starch fraction and polysaccharide gum B-1459 or CMC-7HT (The Hercules Powder Co.) is added at a level of 0.7 to 1.0% based on total solids. The mixture is made up to a ratio of 4 parts of protein to 13 parts of total solids (*2*).

When this mixture is freeze dried, good dispersibility and stability are obtained. Spray drying without further treatment gave good stability but only fair dispersibility. The spray-dried material behaves much like spray-dried nonfat milk, and the instantizing process, as used in the dairy industry, greatly improves dispersibility.

In the laboratory, this is accomplished by spreading a thin layer of spray-dried powder approximately 1/16 inch thick on a metal plate and treating with steam for 30 seconds in an autoclave. The powder is then dried at room temperature. This material disperses reasonably well for practical products. The final product has a slight wheat flavor, but no off-flavors.

Many questions relating to storage stability have not yet been answered. Lipid oxidation can be anticipated as a possible problem. If this problem arises antioxidant might be of value. Since the process minimizes formation of free-amino groups and reducing sugar, sugar-amine browning problems are not anticipated. Nutritional studies have not been made, but the relatively gentle treatment would not be expected to produce substantial nutritional damage Use of wet separation of the acid-soluble material should aid in concentrating the water-soluble vitamins in the product. Also, most of the water-soluble protein is incorporated into the final product. Wheat protein has a somewhat more desirable amino acid distribution than

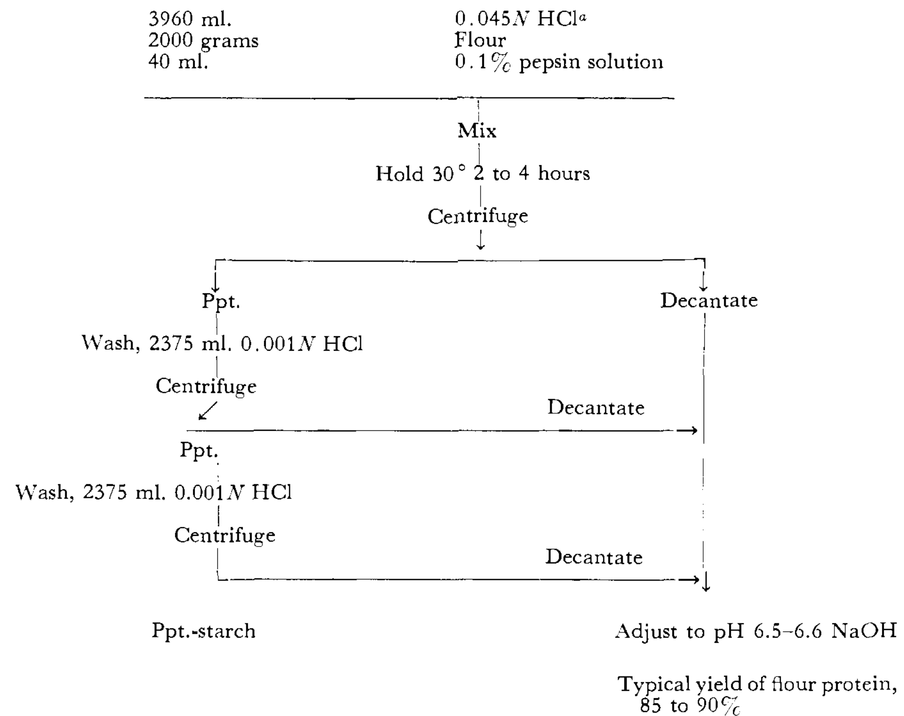

Figure 2. Flow diagram for the wet separation of wheat protein by pepsin-HCl

[a] Adjust HCl concentration if necessary to bring pH below 2.5

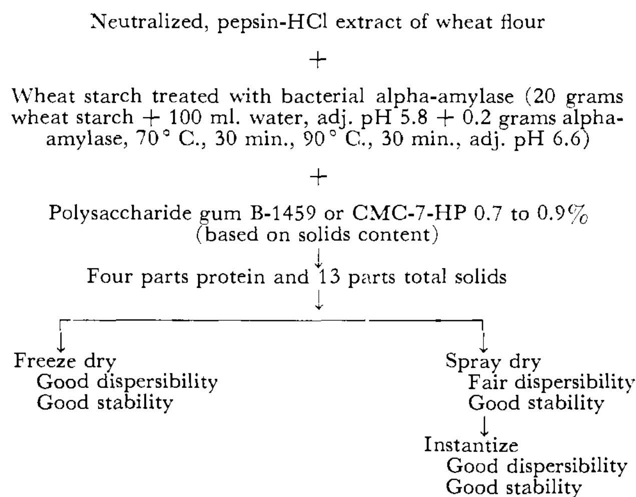

Figure 3. Flow diagram for preparation of a dry milk-like product from wheat

gluten. Additional work is needed on storage stability, nutritional value, and processing costs.

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WORLD-WIDE RESEARCH

Studies on the Chemistry and Biological Effects of Cyclopropenoid Compounds

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"Pink white" and other disorders in eggs arise from the ingestion by hens of leaves and/or seeds of many plants in the order Malvales. The active principles are two cyclopropenoid acids, malvalic (C_{18}) and sterculic (C_{19}), which were isolated and characterized in the fraction giving a positive Halphen color test. When more than 2 mg. of these active acids are fed daily to hens, characteristic disorders develop in the eggs, and are probably caused by greatly increased permeability of membranes within the yolk. The cyclopropene ring structure is essential for biological activity. To aid further biological studies, methods are being investigated for the preparation of cyclopropenoids specifically labeled with C^{14} in the methylene group. Quantitative methods for the estimation of each acid by gas-liquid chromatography have been devised.

STUDIES on the cyclopropenoid compounds form part of the work of the Animal Products Section of the C.S.I.R.O. Division of Food Preservation. The section's work on eggs includes not only disorders induced by diets but also the changes occurring in the whites and yolks of normal eggs during storage. The division's Food Chemistry Section has given considerable help, particularly in those aspects concerned with the physical chemistry of egg proteins and lipids. The division's Plant Physiology Unit has also helped on some aspects of the synthesis of cyclopropenoid compounds in plant cells. This paper comprises a survey of C.S.I.R.O. work on these compounds but is not intended to be a comprehensive review of studies in this field.

Pink White Disorder in Eggs

It has been known for over 40 years that whites and yolks of hens' eggs will discolor during storage if the hens are fed diets containing crude cottonseed products. Sherwood (*34, 35*) showed that they gave rise to two different disorders—a bronze discoloration of the yolk induced by gossypol, and pink whites caused by an unknown factor. Lorenz and coworkers (*19*) showed that seeds of another plant in the family *Malvaceae*, *Malva parviflora*, also induced the pink white condition.

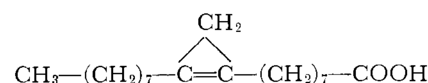
Lorenz (*18*) stated that only those oils giving a positive Halphen color reaction produced pink whites in eggs. The Halphen test (*11*) has been used for many years as an empirical method of detecting the adulteration of more expensive oils by cottonseed oil. This test involves adding to the oil one volume of carbon disulfide containing 1% sulfur, and one volume of pentanol. The carbon disulfide is distilled slowly from an open tube which is heated to 110° C. A pink or red color indicates a positive reaction.

Nature of Active Principle

About 12 years ago, a series of investigations was started on disorders in eggs apparently associated with poultry diets. At that time, there were severe, sporadic outbreaks of the pink white disorder, mainly in eggs produced on the open-range system, and, therefore, a study was commenced on the nature of the active principle causing pink whites.

Guided by Lorenz's observation (*18*) on the association between the Halphen color test and the pink white disorder, the oils from three species of *Malvaceae* were examined—cottonseed (*Gossypium hirsutum*) and the leaves and seeds of two mallows, *Malva parviflora* and *M. verticillata*. In all five oils, a positive Halphen test was always associated with the unsaturated fatty acid fraction. Further fractionation to eliminate oleic, linoleic, and linolenic acids, by methods to be described later, gave an apparently pure substance, with a melting point of 10.3° C. and a molecular weight of 283, and a Halphen color reaction detectable at concentrations down to 10 p.p.m. When fed to laying hens at the rate of about 50 mg. per day, this Halphen-reacting acid produced the typical symptoms of the pink white disorder in stored eggs (*31*).

This biologically active fraction had some of the properties of sterculic acid, the C_{19} cyclopropenoid whose structure, given by Nunn (*25*), is as follows:



After Faure (*9*) reported that sterculic acid gave a positive Halphen test, as-