SOME FACTORS AFFECTING LUMP FORMATION IN FROZEN STARCH-THICKENED SAUCES

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SOME FACTORS AFFECTING LUMP FORMATION IN FROZEN STARCH-THICKENED SAUCES

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ABSTRACT PATRICIA DIANE CUMMISFORD

The frozen-storage stability of white sauce containing a thickening agent, skimmed milk, sodium chloride, and a hydrogenated vegetable oil was studied. Ten thickening agents were included in this study: corn starch, sorghum starch, waxy corn starch, waxy sorghum starch, waxy rice starch, cross-linked waxy corn starch, phosphate cross-linked waxy corn starch, phosphate cross-linked waxy sorghum starch, wheat flour, and waxy rice flour. After frozen storage for periods of one week to six months at -19.5°C., samples were thawed at 35°C. and evaluated by a taste panel and by several physical and chemical tests.

Samples prepared with different thickening agents and stored for different times showed significant differences for several of the factors evaluated by the panel. Most of the sauces were lumpy and were considered unacceptable by the panel after a one-week storage period. Only sauces with waxy rice flour appeared smooth even after a three-month storage period and were acceptable to the panel.

The results of the physical and chemical tests were in agreement with results obtained from the panel. Sauces prepared from all thickening agents but waxy rice flour gave a high degree of separation after only one week of frozen storage. Waxy rice flour sauces separated upon centrifugation only after a three-month storage period. The amount of supernatant from this sample was less than that observed in any of the other samples regardless of storage time. The turbidity of the supernatant from waxy rice flour sauces was greater than that obtained with any of the other sauces stored three months, and was comparable to values obtained with the supernatants from the three cross-linked starches after a one-week storage period. The amount of supernatant from the latter samples was much larger and the sauces were badly lumped.

Samples prepared from glycogen and from nongranular amylopectins from sago, tapioca, and waxy maize starches showed marked variation in stability. Sauces and pastes prepared from glycogen and from tapioca amylopectin were stable to freezing. Average number of glucose residues per end group or average molecular weight of the fractions was not related to stability behavior.

From the study it was concluded that retrogradation of starch was the primary source of instability, with presence of milk proteins being a secondary factor. The tendency to retrograde appeared to be related to structural details of the starch molecules not entirely elucidated by present analytical methods.

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INTRODUCTION

A common binding agent for many food mixtures and the sauces used with vegetables and meats frequently employ a white sauce formula as a base. In recent years there has been much interest in such combinations in the form of frozen meat pies, frozen tray dinners, and frozen specialties such as chop suey, macaroni and cheese, and other gravy-containing mixtures, both for home freezing and for commercial production. The stability of white sauces which were held in frozen storage has been investigated by others who studied effects of viscosity, homogenization, and various stabilizers and emulsifiers on stability (10). Fluctuating temperatures during storage were also studied to determine the effect on the product (11).

The possibilities of future uses for a thickening agent stable to freezing are numerous. As noted in the literature (10), preparation of salad dressings and desserts as well as sauces and gravies which would be stable to freezing would be possible if the right thickening agent or combination of thickening agents could be found. The increased demand for precooked frozen foods seemed to warrant further investigation as to the factors involved in this type of instability.

This investigation was undertaken to study the stability behavior of sauces prepared from ten starches and flours under carefully controlled conditions of mixing, heating, and stirring. Later the study was extended to include the behavior of several pure branched fractions from different starches.

It is hoped that the work described here will provide some useful information on the factors important in the instability of frozen starch-containing sauces and also indicate the need for further information on the molecular structure of starch fractions before complete explanations for the observed behavior can be made.

REVIEW OF LITERATURE

A scientific explanation of the behavior of starch when subjected to various treatments of cooking, aging, and storing typically used with food products has been greatly aided by basic studies of the chemical and physical properties of starches and the constitution of starch molecules and granules. This brief review concerns the observed behavior of starches in foods with respect to various aspects of freezing stability and retrogradation and information on the structure of starch - both granular and molecular - and also on factors which may cause variable results. There is also included a brief section on the constitution of milk and its behavior after freezing.

Instability of Starch to Freezing

Observations on the retrogradation of starch by freezing date back to 1844, according to the review of work in this area presented by Woodruff and MacMasters in 1938 (54). They noted several other accounts of observed retrogradation but pointed out that little information is given in these reports as to the exact temperature, time, or description of appearance of resultant product. Woodruff and Hayden (53) presented photomicrographs showing retrogradation in corn starch

and wheat starch gels. They found freezing at -2°C. gave greater changes than freezing on solid carbon dioxide. Under the conditions used (-2°C.) they found a frozen 5% paste, which had been gelatinized between 75°C. and 95°C., gave a fibrous sponge from which most of the water could be pressed. Veined areas also appeared in the photomicrographs. They suggested that the changes might be related to association of micelles and aggregates formed by dehydration of swollen granules during ice crystal formation. This would allow molecules to draw together through secondary valence forces. Ice crystals which formed in gels frozen in liquid air or solid carbon dioxide would be smaller and would produce less injury to granules. Woodruff and MacMasters (54) stated that gels frozen at -70°C. regained most of the original consistency upon thawing, indicating less chance for orientation at low temperatures. At low temperatures, small ice crystals are formed, thus producing relatively few areas where starch would be free of surrounding ice, (dehydrated areas) affording little opportunity for molecules to become oriented. At higher temperatures of freezing, large ice crystals are formed, causing many areas where starch would be free of surrounding ice, thus providing considerable opportunity for molecular orientation. Woodruff also noted the resemblance of frozen starch gels and cellulose when their fibrous strands were observed with a Spierer lens (52).

Schoch (42) found that the speed of retrogradation depended on the concentration of the paste, 1% pastes retrograding more slowly than 5% pastes. He also found that some modified starches (dextrinized, oxidized, or ethylated) did not retrograde. These observations were not made on frozen samples, however.

These retrogradation changes in starches, especially the behavior when frozen, have been considered serious problems by workers in both food research and food industries. Several suggestions have been made that homogenization or the addition of stabilizers such as gelatin might be used to increase the stability of starch-thickened frozen foods. Hanson, Campbell, and Lineweaver (10) found that the type of thickening agent used was a primary factor in the stability of frozen sauces. They interpreted their results as indicating that amylopectin starches and flours minimized the separation and curdling, although their data did not support this conclusion in all cases. Heating the frozen sauces was found to improve the appearance considerably. This group found that sauces prepared with waxy rice flour were stable after eleven months storage at -18°C. In studies on frozen puddings Hanson, Nishita, and Lineweaver (12) found that puddings prepared with waxy rice flour were stable for six to nine months when stored at 0°F. In the same study, a soft custard-type pudding which contained waxy rice flour was stable for two to four months, while a baked custard preparation of a similar formula was unstable to freezing. A recent paper by Hanson, Fletcher, and Campbell (11) reported that stability of starch-containing mixtures was improved by the addition of pectin, gelatin, certain vegetable gums, and Irish moss extractives. A decrease in egg and in liquid in the formulas and an increase in waxy rice flour and in sugar improved stability. They found temperatures fluctuating between -10°F. and 10°F. more damaging than storage at a constant temperature of 0°F. In white sauces prepared with waxy rice flour, 10°F. storage produced instability after three weeks; storage at fluctuating temperatures of -10°F. to 10°F. produced instability after two months; 0°F. storage produced instability after twelve months, and in samples stored at -10°F., no instability was reported even after thirty-six months frozen storage. They defined stability as less than 3% separation. In samples stored at 10°F., no separation was obtained after a one-month storage period if 1% pectin, 0.4% to 1.5% gelatin, or 0.4% gum tragacanth was added to a waxy rice flour sauce prior to freezing. They indicated that a 1.5% gelatin addition produced a wheat flour sauce stable one month at the 10°F. storage temperature.

Starch Retrogradation

Schoch (42) found an insolubilization of starch when dilute starch pastes were allowed to stand. Retrogradation was suggested as designating "all tendencies of starch to revert to less soluble forms." Schoch and French (47) credit Lindet (19) with the origin of the term retrogradation in reference to the spontaneous formation of crystalline aggregates present in stale bread. Maquenne and Roux (20) later used the same term in referring to insolubilization of starch. Noznick, Merritt and Geddes (36) attributed the staling of bread to branched chains, a concept which was not present in early literature, which attributed staling to the straight chain components. Schoch and French (47) found that the soluble starch in fresh bread was predominately amylopectin and suggested the amylose fraction was insolubilized and retrograded during baking and could not be involved in staling. On standing, the solubles of bread decrease, probably by aggregation of amylopectins into tight lattices involving secondary bonding between branch ends of different molecules. This condition is in contrast to the postulation by Meyer (21) that in strongly swollen starch granules, gigantic amylopectin molecules form a loose three dimensional lattice of interlocked branches free of the secondary bonds that form only slowly during retrogradation. Amylose was not considered to be involved in this behavior. Schoch and French (47) found that stale bread, when leached with water at 50°C., gave a high amount of solubles. The authors noted that this was in agreement with the apparent freshening of stale bread by warming. The retrograded amylose fraction is not solubilized at 50°C. but retrograded amylopectin is. When 50% aqueous wheat starch pastes were prepared and heated so that the temperature rise of the paste over hot water resembled that observed in a loaf of bread during baking, and then allowed to age, similar behavior was noted under these conditions of subsequent extraction. The authors suggested that other bread components may also influence the staling reaction. It was suggested that aggregation of the amylopectin molecules involved an oriented association between terminal branches of these molecules. No aggregation was noted in the absence of free branches as in beta-amylase limit dextrin, starch ethers, or oxidized starches.

Starch Granules

K. H. Meyer and co-workers reported, in a series of articles, their interpretation of the structure and organization of the starch granule. The granule was thought of as a series of concentric layers about a central nucleus (24). The layers were composed of molecules of both branched and linear fractions which were radially oriented. The oriented layers were held together by associations of straight chain molecules and/or the outer branches of the

branched molecules such that microcrystalline areas were formed. These areas were spoken of as "micelles" by Meyer, who used the name given them by Nageli. Within the granule there were areas of crystalline nature interspersed with areas of a lesser degree of orientation, forming a loosely bound network.

In spite of a granule structure which is common for starches, there is wide variation in the behavior of starch isolated from different species, varieties, and types of plants (48). These properties are also influenced by growing conditions and isolation operations (17). Thus, in working with starches it is desirable to correlate several properties determined with one specific sample rather than to determine one property with one sample and another property with a different sample.

Starch Fractions

For many years it was suspected that the granules contained more than one main type of component. In a recent review of the starch fractions (46), it was reported that van Leeuwenhoek (55) in 1716 found, by microscopic studies, that a portion of the granule was insoluble even when the granule was heated in water. Other early investigators, including Guerin-Varry (7) described three components, based on their solubilities in hot and cold water; this observation was not substantiated

by later findings (34).

It was not until late in the nineteenth century that an understanding of the nature of starch was furthered by C. W. Nageli (35). Although his concept of the similarity of the insoluble component to cellulose, with the soluble fraction being merely a physical modification of the insoluble fraction, has more recently been proved incorrect, he did contribute to our present basic knowledge, especially with respect to the micellar structure of granules.

Maquenne and Roux (20) made the first serious attempts to fractionate starch; a fraction which they called amylocellulose, actually the one which ultimately became known as amylose, was precipitated by allowing the centrifugate from starch pastes or autoclaved sols to stand at room temperature. Malt conversion of the precipitate gave 96-98% yield of maltose. They believed malt ineffective with amylopectin, and concluded starch consisted of 18-20% amylopectin since starch gave only 80-82% conversion to maltose. Amylopectin was not isolated by these workers, but they were the first to recognize the existence of two chemical fractions of starch.

Indeed, much of the early work on fractionation resulted in fractions with varying degrees of purity (48), leading to confusion as to interpretation of results.

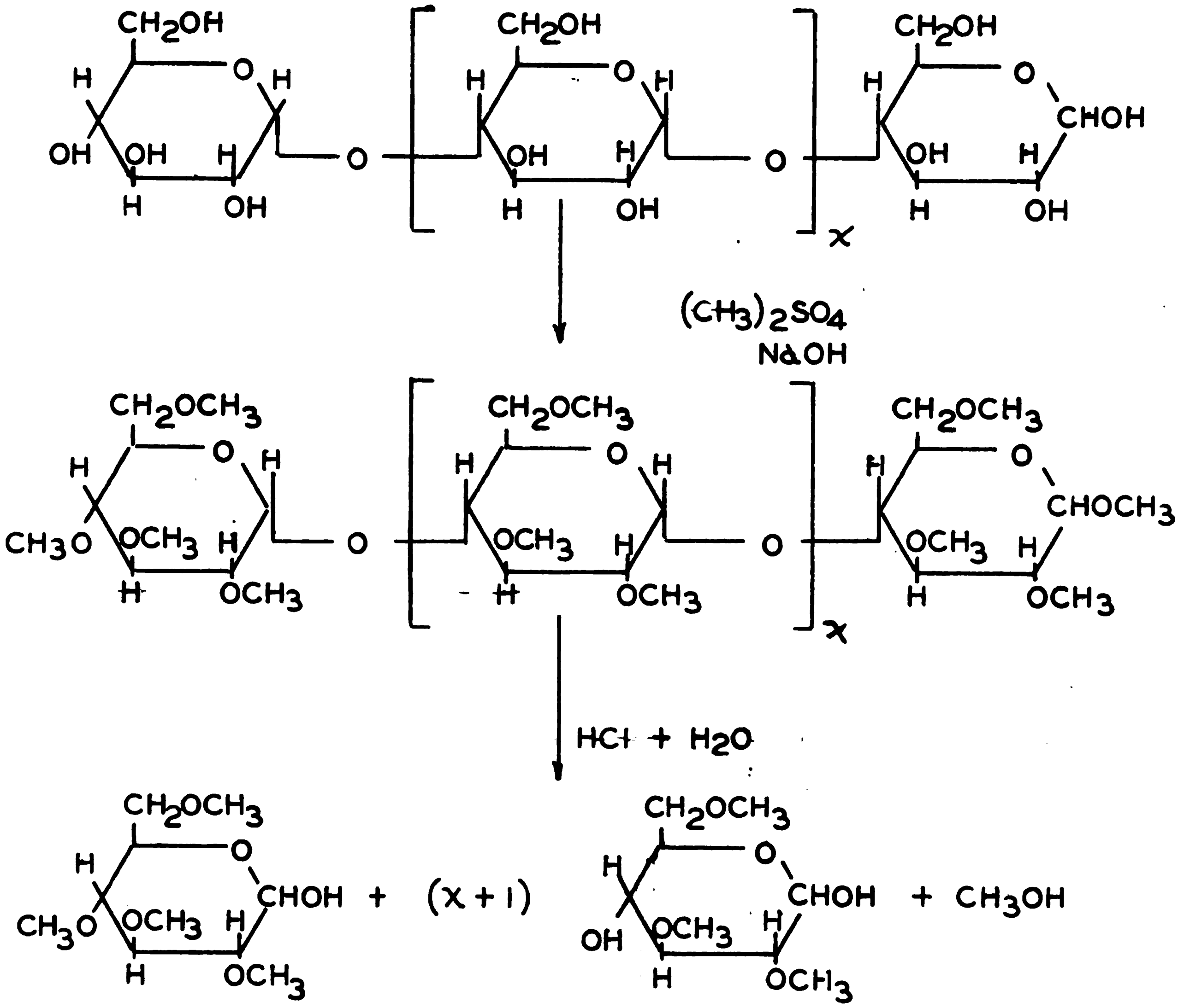
It was not until 1941 and 1942 that Schoch (42, 43) reported the use of butyl or amyl alcohol to selectively precipitate the component soluble only in hot water, which gave an irreversible (retrograded) gel on cooling. The easily soluble fraction could then be precipitated by treatment of the solution with methanol.

The naming of starch fractions has varied considerably, being especially confusing in the early literature. K. H. Meyer (26) suggested amylose and amylopectin for the linear and branched components respectively. Later the terms A- and B- fraction were introduced by Schoch for these respective components (44). He later suggested the descriptive terms "linear starch fraction" and "branched starch fraction" (45).

Amylose

The structure of starch has been investigated by several methods. For amylose the bulk of evidence points to a linear polymer of glucose units linked by 1,4 - glucosidic bonds of the alpha configuration. Haworth, Hirst, and Webb (14) found that potato starch, when completely methylated and then hydrolyzed, gave a large amount of 2,3,6 - trimethylglucose and a small amount of 2,3,4,6 - tetramethylglucose. The 2,3,4,6 - tetramethylglucose is derived from the terminal nonreducing end of the molecule, while all other glucose residues give the

2,3,6 - trimethylglucose. This may be shown for amylose:



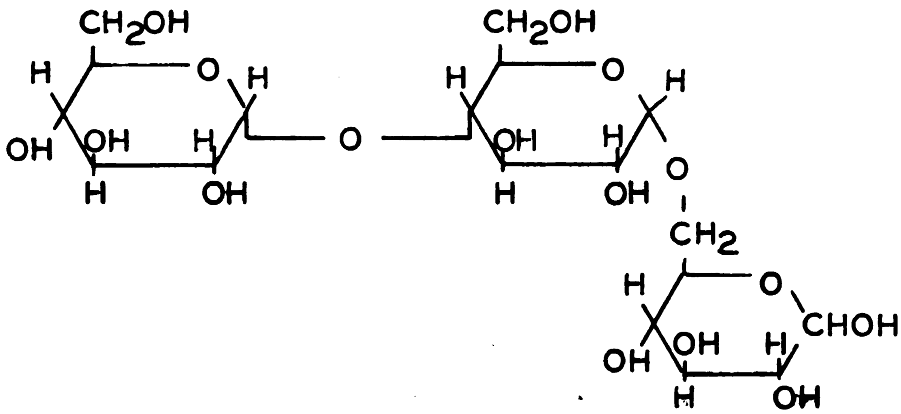
2,3,4,6-TETRAMETHYLGLUCOSE 2,3,6 TRIMETHYLGLUCOSE

Such methylation studies by Meyer and co-workers (32) indicated amylose was a linear polymer of three hundred glucose residues. The alpha configuration of linkages in amylose was substantiated by enzyme studies with beta-amylase. It was found (26) that amylose was completely converted to maltose by this enzyme, indicating 1,4 - glucosidic linkage of the alpha configuration. Meyer and Bernfeld (25) found that amylose gave a pure dark blue color with iodine solutions. The intense blue color was not found to be characteristic in general of the branched fraction of starch and of glycogen.

Amylopectin

Methylation, periodate oxidation, and enzymatic methods have been used in the characterization of the two branched polysaccharides amylopectin and glycogen. Freudenberg and Boppel (6) found 2,6 - and 2,3 - dimethylglucose present after hydrolysis of completely methylated potato starch, in addition to the tri- and tetramethylglucose components observed with amylose. They thought the 2,6 - dimethylglucose arose from hydrolysis of the other compounds but suggested that 2,3 - dimethylglucose resulted from methylation of residues linked at points of branching. From this it is concluded that branching occurs at position 6. Montgomery, Weakley, and Hilbert (33) isolated isomaltose from enzymic hydrolysates of starch. The structure of the disaccharide was proved to be 6-alpha- D-glucopyranosyl-D-glucose from a comparison of its octaacetyl derivative with the same derivative of 6-alpha-D-glucopyranosyl-beta-D-glucose isolated from dextran hydrolysates. These octaacetyl derivatives were identical. Further evidence for the existence of 1,6 - glucosidic linkages in amylopectin, and for the alpha configuration of these linkages, is presented by Thompson and Wolfrom (49). They isolated panose, as a derivative, upon the acid hydrolysis of

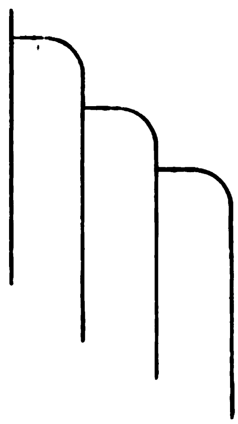
amylopectin. Panose,



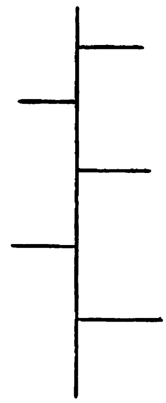
4-α-ISOMALTOPYRANOSYL-D-GLUCOSE
contains both 1,4 and 1,6 bonds of the alpha configuration.

That amylopectin differed from the straight chain structure of amylose was also shown by enzymic studies. Meyer, Bretano, and Bernfeld (26) found that amylopectin could not be completely saccharified by beta-amylase treatment but rather formed dextrins which could be further degraded by beta-amylase only after treatment by alpha-glucosidase (23), specific for alpha configuration at 1,6 - glucosidic linkages. These results did not agree with the schematic formula for amylopectin suggested by Staudinger. Later evidence also suggested that amylopectin was a multiply branched molecule. Peat, Whelan, and Thomas (38) and Larner and co-workers (18) concluded that of three schematic representations presented, Meyer's representation was the only one which could give the yields of maltose and maltotriose obtained.

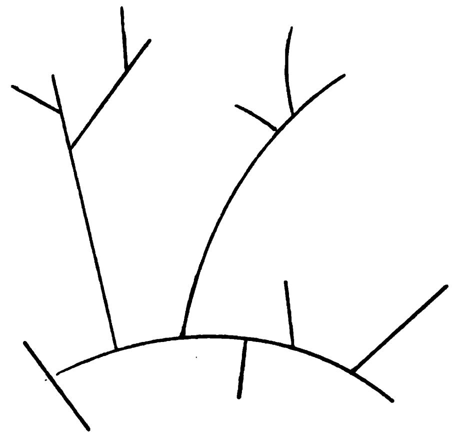
The three proposed structures were:



HAWORTH'S LAMINAR STRUCTURE



STAUDINGER'S COMB STRUCTURE



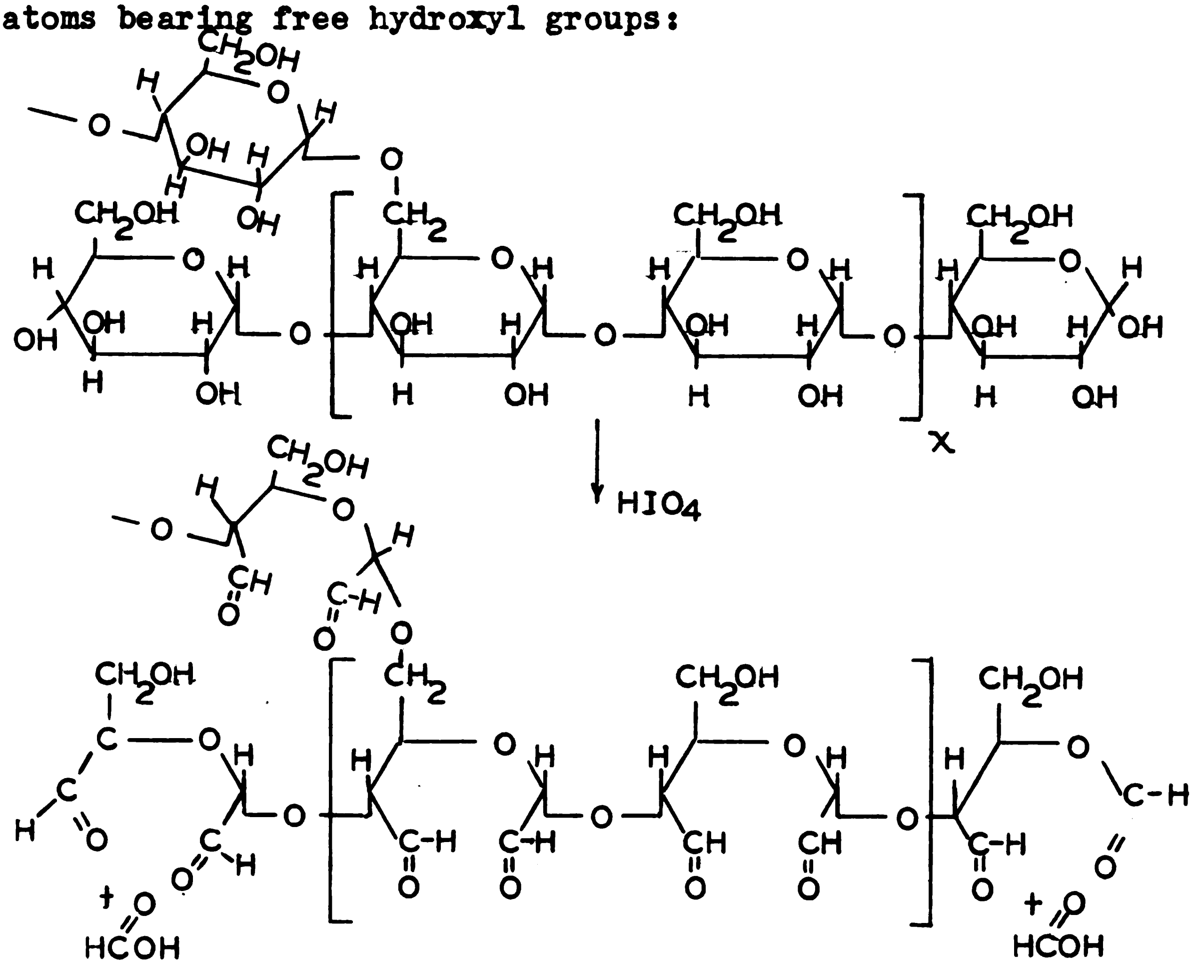
MEYER'S ABORESCENT STRUCTURE

Larner, Illingworth, Cori, and Cori (18) found that amylopectin samples they analyzed contained at least five tiers of branches, and that the number of branches per tier decreased as the reducing end of the molecule was approached. In earlier studies, Cori and Larner (4) had found that the ratio of free to phosphorylated glucose was characteristic of the polysaccharide studied by successive phosphorylase and amylo-1,6-glucosidase action. Peat and co-workers (39) concluded that Meyer's representation was correct since successive beta-amylase and debranching enzyme treatments were necessary to degrade amylopectin completely.

The third method commonly employed in studying the constitution of amylopectins was periodate oxidation. Jackson and Hudson (15, 16) reported their basic work on the topic in 1936 and 1937. In 1945, Brown and co-workers

(2) reported the agreement of periodate data and methylation values as to average chain length.

In 1948, Potter and Hassid (40) determined end-groups in amylose and amylopectin samples which were carefully purified and defined as to source (17). The reaction involved a splitting occurring between adjacent carbon atoms bearing free hydroxyl groups:



Usually, free formic acid is determined by titration and the number of nonreducing end groups is calculated from this value. The method has been modified by several people, and recently Hamilton and Smith (8) used a sodium borohydride reduction to form polyalcohols from the polyaldehydes present after treatment with periodate.

After hydrolysis, the mixture of glycerol (derived from terminal residues) and erythritol (derived from nonterminal residues) could be examined for relative quantities of each. From the results they obtained, it was concluded (9) that free glucose should be obtained after amylopectin is subjected to the described treatment only if bondings other than alpha 1,4- and alpha 1,6- glucosidic linkage were present. Occasional observations have suggested 1,5 (1) and 1,3 linkages (51), but there is no general agreement on this matter, the bulk of evidence pointing to the other types of linkage as the principal ones.

The structure of glycogens has been studied in many cases concurrently with amylopectin (30, 18). The results indicate that glycogen resembles amylopectin generally, but the former is more bushy and more highly branched with shorter branches, both inner and outer. Cori (3) has found glycogen to possess at least ten tiers of branches.

Meyer and Heinrich (28, 29) could find no amylose present in certain starches, namely waxy rice and waxy maize starches. The former was described as having properties approaching those of glycogen. Later it was noted (31) that much variation occurred in fractions obtained from tapioca and from waxy maize amylopectins with respect to percent end groups and susceptibility to enzyme degradation.

In enzymatic studies with amylopectin and glycogen, Meyer and Fuld (27) and Meyer (22) reported that amylopectin gave 15 to 18 glucose units per outer branch, 8 to 9 per inner branch, and 4% branching. Glycogen gave 6 to 7 glucose units per outer branch, 3 units per inner branch, and 9% branching. From the results it was concluded that glycogen was unable to form insoluble crystalline regions because of shortness of outer branches.

Several specific amylopectins have been studied in detail. Potter and Hassid (40) using purified amylopectins which had been prepared and studied by Lansky, Schoch, and Kooi (17), found these fractions had between 22 and 27 residues per end group according to data obtained from periodate oxidation. Hassid (13) later found waxy rice starch gave a value of 20 residues when the same technique was employed. Molecular weights of these fractions were also determined by osmotic pressure measurements of acetylated amylopectins (41). They obtained the following results:

Source	Code	Residues/end group	Molecular Weight
Sago	S-1-B	22	1,000,000
Corn	C-109-B	25	5,000,000
Corn	C-141-B	26	6,000,000
Wheat	W-2-B	23	4,000,000
Easter Lily	L-3-B	27	3,000,000
Tapioca	T-3-B	23	3,000,000
Potato	P-3/4-B	27	———
Waxy Rice	———	20	———

Milk and the Reaction of Milk to Freezing

Webb and Hall (50) noted that after freezing a hydrophilic sol will repeptize upon thawing while a hydrophobic sol precipitates when thawed. Casein, a weakly hydrated protein, may disperse. The destruction of the caseinate colloid involves long storage times and there is a progressive decrease in dispersion of casein with frozen storage. Denaturation of casein proceeds in a limited but progressive manner. Skimmed milk when frozen at -18°C. showed distinct casein separation at 12 weeks and at 17 weeks a clear serum separated by thawing at room temperature.

The protein content of skimmed milk is generally given as between 3.4 and 3.8% and the protein content of whey is about 0.85% (5).

PROCEDURE

Preliminary Investigations

A preliminary investigation of several factors was made before an attempt was made to set up the experiment in final form. Since carefully controlled cooking conditions were desired, the effects of preparing the sauces in the Corn Industries Viscometer were determined by comparing standard white sauces prepared using this instrument with standard white sauces prepared over a boiling water bath with manual stirring. Not only were the temperature increments similar for the sauces prepared by the two methods, but also the viscosities of the sauces prepared by the two methods were comparable. In determining viscosity of the prepared sauces, a MacMichael Viscosimeter was used. Under conditions of the test (number 28 wire, sauce at 68°C., and water bath at 68°C.), the range of readings for sauces prepared in the viscometer was 14 to 17 scale units and the range for sauces prepared over boiling water was 13 to 19 units. The readings indicate the ranges obtained on duplicate determinations with each of two sauces for each method. The sauces stirred manually showed readings of 13 to 16 on two determinations from one batch of sauce and 15 to 19 on two determinations of the other batch. Sauces prepared in the viscometer showed the same reading (14 to 17) for the four determinations. Upon thawing after freezing, sauces prepared by both methods showed lumpiness. Microscopic examination of the sauces also indicated similarity, both before and after freezing. On the basis of these results, it was decided to prepare the experimental samples in the viscometer.

The ratio of ingredients used by Hanson (10) was originally considered for use in this experiment. It was found, however, that with the amounts of thickening agents necessary to give final hot paste viscosities comparable to that obtained with wheat flour the fat was not emulsified in all instances. The sauces were cooked five minutes after maximum viscosity was reached, or, with those thickening agents showing no maximum, ten minutes after the start of the increase in viscosity was observed. Because of the difficulty in fat emulsification, the amount of fat was decreased to the smallest proportion which would still allow easy blending of the large amount of flour which was used. The sauces were prepared so that the amount of thickening agent used would give a final viscosity of about 30 gram-centimeters.

In order to measure the amount of liquid separated from the thawed samples by an objective method, it was necessary to find a method which would precipitate the lumps present in the thawed samples but would not bring about separation of freshly prepared sauces. In tests on frozen samples prepared with various thickening agents, it was found the flocculent material of the frozen samples was precipitated by a Sorvall Superspeed Angle Centrifuge run at 6100 r.p.m. (12,200 G. force) for 15 minutes. This same treatment caused no separation in either the unfrozen sauces or the skimmed milk used in preparing the sauces.

It was also noted that the supernatant obtained on centrifugation of the thawed samples varied greatly in turbidity. Investigation showed that differences between the supernatants could be distinguished by reading percent transmission of light using a distilled water blank as 100% transmission. The test samples were diluted with distilled water to obtain samples in the readable range. A Bausch and Lomb Spectronic 20 Colorimeter was used. The wavelength of 625 millimicrons was chosen for the tests since this wavelength most effectively eliminated the absorption of light due to the presence of yellow-green components of whey and flour pigments.

Preparation of Samples in the Viscometer

Sauces were prepared using ten thickening agents. The preparation pattern consisted of seven randomized blocks. The seven blocks were prepared over an eleven-day period, with each block being completed within an eighteen-hour time.

The ingredients used in the sauces were each obtained from a single source or lot with the exception of the skimmed milk, which was obtained from the Michigan State

Creamery in four lots. The processing of the milk was controlled so that the four lots would be as nearly alike as is possible in a natural product such as milk. The first three blocks were prepared from the first two lots of milk and of the remaining four blocks two were prepared with each of the two remaining lots of milk.

The fat used was a hydrogenated vegetable oil (Crisco, lot 076A6).

Chemically pure crystalline sodium chloride (Malinkrodt Analyzed Reagent) was used for the salt ingredient.

The thickening agents used in the experiment were each obtained from a single lot. The sources of the various thickening agents are given in Table I.

The "dry" ingredients were weighed onto smooth, lightly waxed paper. A stainless steel spatula was used in transferring the materials to the paper from the containers in which the ingredients were stored. A Harvard trip balance was used, and the weighings were made to one-tenth of a gram.

The milk was weighed in a flexible polyethylene bowl. Due to the large amount used, a larger direct-reading balance was used.

The amounts of thickening agents used per batch of

TABLE I

AMOUNTS OF THICKENING AGENTS USED PER BATCH OF SAUCE[a] OR PASTE[b]

Thickening Agent	Grams
Waxy Corn Starch American Maize-Products Company	21.1
Waxy Rice Starch Isolated from waxy rice flour by Dr. S. A. Watson	21.5
Waxy Sorghum Starch Corn Products Refining Company	22.0
Phosphate Cross-linked Waxy Corn Starch American Maize-Products Company	36.1
Phosphate Cross-linked Waxy Sorghum Starch Corn Products Refining Company	37.0
Waxy Rice Flour Rice Products Company	37.5
Sorghum Starch Corn Products Refining Company	38.0
Corn Starch Corn Products Refining Company	40.0
Cross-linked Waxy Corn Starch National Starch Company	41.5
Wheat Flour Gold Medal Brand, purchased locally	58.0

[a] 1082 grams skimmed milk
7.2 grams sodium chloride
50.0 grams hydrogenated vegetable oil

[b] 1082 grams distilled water

sauce are given in Table I. The amounts of fat, sodium chloride, and skimmed milk remained constant per batch for all thickening agents: fat, 50.0 grams; sodium chloride, 7.2 grams; and, milk, 1082 grams.

The fat was liquified by warming slightly in an aluminum sauce pan and the thickening agent blended with it to form a roux. Approximately three-fourths of the skimmed milk, which had been heated to 60°C., was added. The sodium chloride was then added and the mixture was stirred to dissolve the crystals. The mixture was then transferred to a one-liter Florence flask for pouring into the cooking beaker of the viscometer, the stirrer of which was started before adding the mixture. The mixing utensils were washed with the remaining warm milk which was then added to the mixture in the viscometer. The viscosity recorder was started and the temperature of the mixture was recorded each minute.

Throughout the cooking, the water-glycerol bath surrounding the stainless steel cooking beaker was maintained at 100°C. plus or minus 0.5°C. It was occasionally necessary to add additional water-glycerol mixture to maintain the level of the bath such that the beaker would be immersed to the proper level. The stirring speed was maintained at level two, with the scraper turning 24 r.p.m. clockwise and the agitator turning 60 r.p.m. counterclockwise.

The cooking was considered completed five minutes after maximum viscosity, with those thickening agents showing a maximum viscosity, or, in those showing no maximum viscosity, at a point where the increase in viscosity per time unit was small, about ten minutes after the start of the rise in viscosity.

The recorder and the stirrer were then stopped, the stirrer was detached, and the beaker was removed from the cooking bath. The contents of the beaker were transferred to a one-quart Pyrex glass measuring cup to cool the sauce and thus prevent further cooking, and to facilitate pouring. Any fat which was not emulsified was skimmed off and, in the case of slight lumping which occurred consistently in sauces prepared from one of the thickening agents, the sauce was strained through a kitchen strainer.

Lacquered 5-Z short tins were tared and filled with 120 grams of sauce. Immediately after filling, each can was sealed using a hand operated can sealer. The cans were initially cooled in an ice bath, being transferred to a chest-type freezer after the eight cans from a batch were sealed. The cans were identified with code numbers indicating the block and the thickening agent. The cans were held in the quick-freeze portion of the freezer overnight and then transferred to the storage portion of the freezer. The freezer was maintained at -19.5°C. plus or minus 0.5°C.

throughout the three-month storage period during which subjective and objective evaluations of samples were made periodically. Samples remaining at the conclusion of the three-month storage period were packed in dry ice after four months and transported from East Lansing, Michigan, to Urbana, Illinois, where they were held in frozen storage for two months after which objective examinations were carried out.

In order that the involvement of the thickening agents in the instability problem could be ascertained, pastes were prepared using 1082 grams distilled water with the amounts of thickening agents used in the sauces. In preparing these, the procedure resembled that used in preparation of sauces. However, the thickening agent was blended with the water and the mixture cooked to 95°C. (40 minutes) since the viscosity was not sufficient to activate the recorder. Also, only four instead of eight cans were frozen for each batch. After one week these samples were thawed for use in objective tests.

Evaluation by the Panel

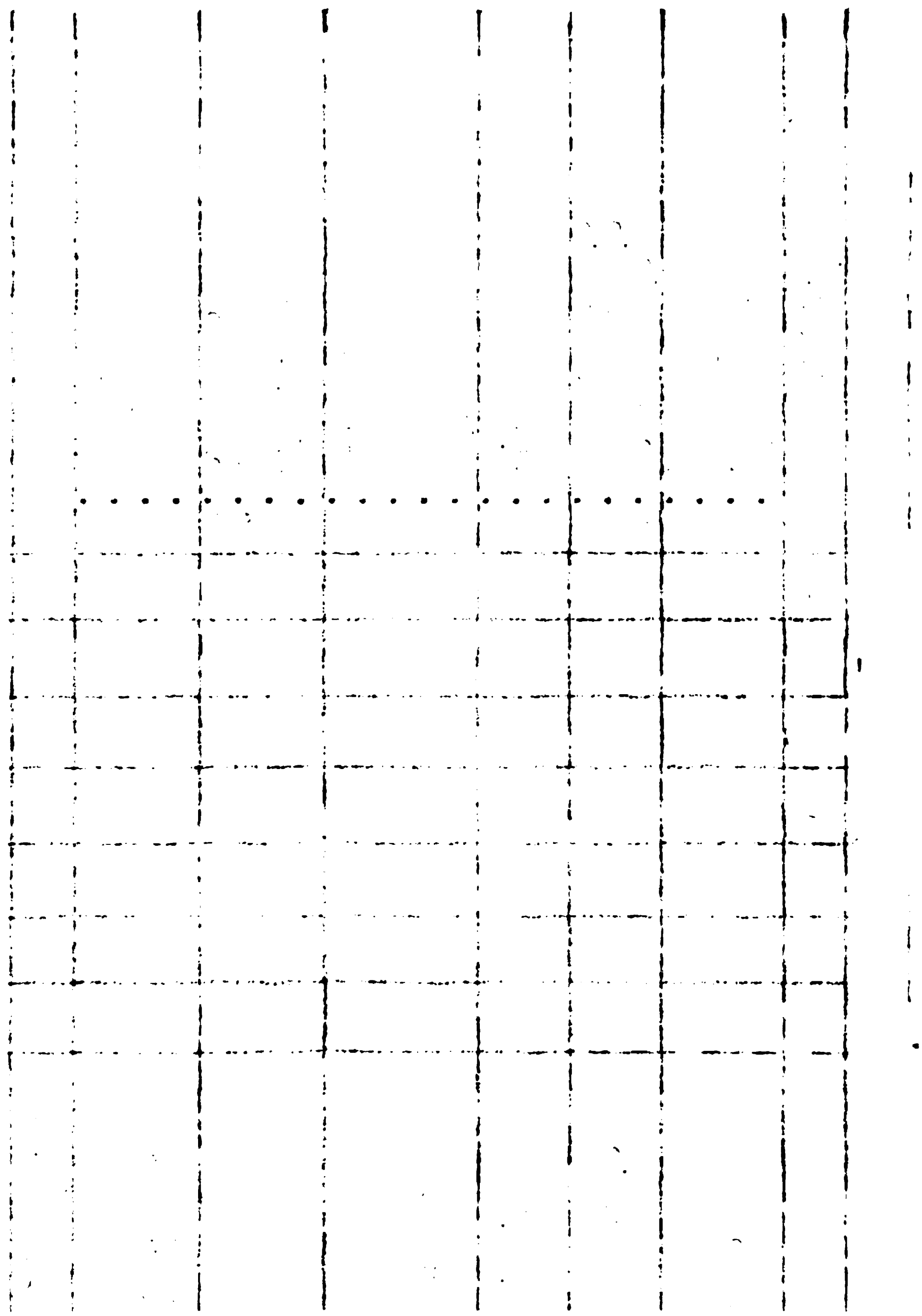
A five member panel composed of faculty and staff members and graduate students from the Department of Foods and Nutrition evaluated the sauces using the score card illustrated in Figure 1.

The storage times included in the study were one week, Sample No. ______ Judge ______

SCORE CARD - WHITE SAUCE

	7	6	5	4	3	2	1	DESCRIPTION (circle most appropriate term)
Smoothness								a. homogeneous b. small grains c. large lumps d. flaky
Separation								a. no separation b. some separation c. much separation
Separated Liquid								a. none b. watery c. milky
Mouth Feel								a. velvety b. pasty c. sandy d. lumpy e. slippery
Flavor								a. bland b. raw cereal c. salty d. off flavor
Paste Character								a. short b. gummy c. stringy d. friable
General Acceptability								

Figure 1. Score card used by panel in evaluating samples.



one month, and three months. For each storage time, the panel was scheduled on two consecutive days. Four replications were used. Samples were presented in duplicate from one block on the first day and on the second day were presented in duplicate from another block. Samples were presented in two groups for each replication, five frozen samples being presented with a wheat flour sauce freshly prepared to serve as a comparison. During the four replications, frozen sauces were grouped so that each thickening agent was presented with every one of the others at least once, so that all possible comparisons were made.

In preparing the samples for panel evaluation, duplicate samples were removed from the freezer and the cans immersed in a 35°C. water bath for one hour. The cans were then opened and the contents of one of the cans was placed in an open Pyrex Petri plate (15 centimeters diameter) while the contents of the duplicate can was divided among five Pyrex watch glasses (10 centimeters diameter). The control sauces were similarly prepared. The samples in Petri plates were placed on a black background to provide contrast to aid in evaluation of smoothness, amount of separated liquid, and characteristics of the separated liquid. The samples on watch glasses were arranged on white enameled trays. The panel members were asked to evaluate the remaining factors from these

individual samples.

All samples including the freshly prepared sauce were identified by code numbers only. All scoring was done in a room provided for that purpose.

The data obtained from the panel evaluation of the frozen samples was analyzed using an analysis of variance. In the method of analysis employed, the error terms used in calculating F values consisted of pooled significant interaction errors. Thus, in calculating the F value for "Thickening Agents," all significant second-order interaction sums of squares involving thickening agents were combined into a new error term.

Evaluation by Objective Tests

The samples on which the physical tests were performed were thawed in the same manner as those used in the subjective evaluation. Two samples were centrifuged simultaneously using three stainless steel centrifuge tubes per sample. The tubes were closed with stainless steel pressure caps, placed in a Servall Superspeed Angle Centrifuge, and the top of the centrifuge securely closed. The centrifuge was then gradually brought to a speed of approximately 6100 r.p.m. (a force equivalent to 12,200 G.). The speed was controlled by means of a rheostat which had been calibrated so that certain points on the rheostat control

allowed a known maximum speed. The centrifuge was maintained at the desired speed fifteen minutes, after which it was gradually stopped. The caps were removed from the tubes and a one-milliliter aliquot of the supernatant from one tube of each sample was transferred to a nine-milliliter distilled water blank in a stoppered test tube. The remainder of the supernatant was decanted into a hundred-milliliter graduated cylinder and the volume recorded.

The diluted aliquot of the supernatant was used in the determination of turbidity. A Bausch and Lomb Spectronic 20 Colorimeter set at a wavelength of 625 millimicrons was used in these determinations. The instrument was adjusted so that distilled water gave 100% transmission. Optically matched glass tubes were used for both blank and samples. The instrument was frequently checked using the distilled water blank. In certain cases a further dilution was needed. One-milliliter aliquots of the appropriate samples previously diluted 1 to 10 were added to nine-milliliter distilled water blanks in the necessary instances. All aliquots and blanks were measured using calibrated pipettes of appropriate sizes.

The objective tests were run on two consecutive days and coincided with the panel evaluation. One sample of each thickening agent was centrifuged each day and turbidity of the supernatant was determined. Checks were run if values obtained on duplicate samples varied more than five milliliters in the amount of supernatant, and whenever time permitted, the values for turbidity were checked if they varied by 10%.

The objective tests were also run on samples stored six months which were thawed in a water bath at 100°C. for 45 minutes and then allowed to equilibrate at room temperature for 15 minutes before centrifugation.

Determination of Nitrogen Content of the Supernatant Liquids

In order to determine whether the differences in turbidities were due to the presence of starch or of protein, Kjeldahl nitrogen determinations were made on aliquots of the supernatants from samples of one of the blocks which had been stored one month. At the same time the nitrogen in the supernatant from a sample prepared from wheat flour and distilled water and held frozen three weeks was determined in order to correct the wheat flour supernatant nitrogen value of sauces for the soluble nitrogenous material from the flour.

The nitrogen determinations were carried out by a modified Kjeldahl method currently used in the Chemistry Department of Michigan State University. Duplicate aliquots were used from supernatants of sauces prepared from all thickening agents with the exception of waxy rice flour which gave no separation on centrifugation after a one-month period of frozen storage. Duplicate aliquots of a solution of ammonium sulfate, which contained a known quantity of the compound, were used as standards. Five-milliliter aliquots of sample were pipetted into dry five hundred-milliliter Kjeldahl flasks. To each flask 0.3 gram cupric sulfate, 10 grams potassium sulfate, and 25 milliliters concentrated sulfuric acid were added. The flasks were placed in digestion racks and heated for one-half hour after the contents became a clear blue-green color. The flasks were allowed to cool in the racks and then 175 milliliters of cool tap water was added to each flask. Granulated zinc was added to the cool flasks and 75 milliliters of 40% sodium hydroxide was added by pouring it down the side of the flask so that the sodium hydroxide would collect in a layer at the bottom of the flask and would not mix with the contents of the flask. The flasks were then connected with the distillation columns and the contents of the flask mixed by swirling. The flasks were heated and the distillate was collected in 50 milliliters of 4% boric acid solution contained in a 500-milliliter Erlenmeyer flask. The boric acid solution contained ten drops methyl red indicator and two drops 0.05% methylene blue solution. The distillation was stopped when the boric acid plus distillate totalled approximately 225 milliliters.

The contents of the receiving flasks were then titrated with standard 0.1052-N. hydrochloric acid to the end point as judged by comparison with a reference solution prepared by placing 50 milliliters of 4% boric acid solution, 175 milliliters distilled water, ten drops methyl red indicator, and two drops 0.05% methylene blue solution into a 500-milliliter Erlenmeyer flask. This solution is violet colored and has a pH of approximately 5.0.

The amount of ammonium sulfate in the standards and the protein equivalent of the samples were calculated. The factor used in calculating percent protein content was that given for milk proteins by the Association of Official Agricultural Chemists (37): 6.38 times percent nitrogen in the sample.

Preparation and Evaluation of Additional Samples

Since all of the thickening agents with the exception of waxy rice flour produced sauces exhibiting marked instability with as little as a one-week period of frozen storage, a further investigation of possible contributing factors was made. In order to determine the effect of a highly ramified structure on stability in frozen storage, glycogen was selected for investigation. Several non-granular samples of amylopectins were also included to determine whether granular structure was a contributing factor and whether all amylopectins gave similar results.

Several of the thickening agents used previously were also included as checks.

Five percent of the amounts of ingredients used in the viscometer preparations was used in preparing the corresponding small samples. Two grams was the weight arbitrarily chosen for the amylopectins and glycogen. Amounts of thickening agents used are recorded in Table II. In the preparation of these samples, 2.5 grams of fat was melted in a large test tube in a boiling water bath. A roux was made with the thickening agent, and 54.1 grams skimmed milk was added and mixed, using a thermometer to stir the mixture. After 0.36 gram sodium chloride was added, the mixture was cooked to 95°C. in a boiling water bath. Twenty grams of the sauce was weighed into each of two size one porcelain crucibles and the remaining portion was poured into a third crucible. The crucibles were covered with porcelain lids and wrapped in polyethylene film before freezing to prevent excessive dehydration. All three samples were frozen. The last poured, containing less than twenty grams of sauce was thawed after only one day of frozen storage and examined macroscopically.

Skimmed milk was also heated to 95°C. and samples were frozen. Skimmed milk, fat, and sodium chloride mixtures were similarly prepared. The various thickening agents were also prepared with distilled water by making a

TABLE II

AMOUNTS OF THICKENING AGENTS USED PER BATCH OF SAUCE[a] OR PASTE[b]

Thickening Agent	Grams
Waxy Corn Starch	1.06
Waxy Rice Starch	1.08
Phosphate Cross-linked Waxy Corn Starch	1.80
Waxy Rice Flour	1.81
Corn Starch	2.00
Glycogen (Pfanstiehl)	2.00
Tapioca Starch Amylopectin Batch T-8-B (T. J. Schoch)[c]	2.00
Sago Starch Amylopectin Batch S-2-B (T. J. Schoch)[c]	2.00
Waxy Maize Branched Material Batch WM - 1/2 - B (T. J. Schoch)[c]	2.00

[a] 54.1 grams skimmed milk
0.36 gram sodium chloride
2.5 grams hydrogenated vegetable oil

[b] 54.1 grams distilled water

[c] For fractionation procedure see S. Lansky, M. Kooi, and T. J. Schoch, Properties of the Fractions and Linear Subfractions from Various Starches. J. Am. Chem. Soc. 71: 4066-4075 (1949).

slurry and heating it to 95°C. In all instances duplicate preparations were made with two twenty-gram samples being frozen from each. The remaining paste from each batch was poured into a third crucible. This sample was then stored frozen for two days, at which time macroscopic evaluations were made.

Half of the twenty-gram samples were stored frozen for one week and then examined and evaluated by the same objective tests that had been used on the former samples. The crucibles were removed from the freezer and allowed to thaw at room temperature (27°C.) for two hours. The contents of each crucible was transferred to a stainless steel centrifuge tube. Subsequent centrifugation and turbidity measurements were the same as previously described. The remaining samples were stored at -19.5 plus or minus 0.5°C. for four weeks after which they were packed in dry ice for transporting from East Lansing, Michigan, to Urbana, Illinois, for two months additional storage.

RESULTS

Observations of Sauces during Preparation

Although precautions were taken to make the preparation of all the sauces as nearly the same as possible, differences in the ingredients themselves introduced slight variations in procedure. There was some variation in the time required to combine the ingredients, since certain thickening agents were more readily blended with the fat and milk than others. However, the total time was consistent for all batches made with one thickening agent.

In general, each thickening agent followed a consistent pattern during cooking with respect to temperature increments with time, total time required to reach the end point of cooking, final temperature of the mixture, and viscosity pattern. There was some variation in the last mentioned property occurring primarily with one batch of milk. The temperature of the mixture in the cooking beaker rose steadily to a maximum value with all thickening agents with the exception of wheat flour white sauces. These showed a rise in temperature initially, followed by a fall and subsequent rapid rise to the final value. The fall in temperature occurred simultaneously with the start of the rise in viscosity. The drop and subsequent rise to the former high value occurred within a two-minute interval.

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There was no comparable occurrence in the waxy rice flour samples or in the wheat flour paste prepared with distilled water.

Samples prepared from phosphate cross-linked waxy corn starch consistently showed a slight lumpiness which necessitated straining.

In spite of the relatively lean formula used, the various sorghum starches formed sauces which showed considerable unemulsified fat. The unemulsified fat was greater in amount with sorghum starches than with the corresponding corn starches. Sorghum starch sauce had a layer of fat on top; corn starch had a lesser amount. Waxy sorghum starch sauces had considerable unemulsified fat; waxy corn starch sauces had a slight amount. Phosphate cross-linked waxy sorghum starch sauces had a slight amount of unemulsified fat; modified waxy corn starch sauces in general showed no unemulsified fat.

Observations of Sauces after Freezing and Thawing

At the one-week storage time the samples prepared from wheat flour showed considerable lumping, as did waxy corn starch and waxy sorghum starch sauces. The corn starch and sorghum starch sauces became rigid and had a spongy texture which exuded a watery liquid when cut. Increased storage times merely intensified the changes in these samples.

After a one-week period of frozen storage, sauces prepared from certain of the thickening agents were relatively free from lumping and had very little if any watery liquid separated. The samples which behaved in this manner included sauces prepared with waxy rice flour, waxy rice starch, and the three derivatized starches. Longer periods of frozen storage caused marked deterioration in all of these samples. At the end of the one-month storage period, waxy rice flour and waxy rice starch sauces appeared superior to the others, which were lumpy and sandy but showed no considerable change in the apparent liquid separation. After a storage period of three months, all samples were definitely changed. Waxy rice flour sauces showed a slight sandiness but no visible separation, while the others were badly lumped and separated. Figure 2 illustrates differences apparent after the one-month storage period.

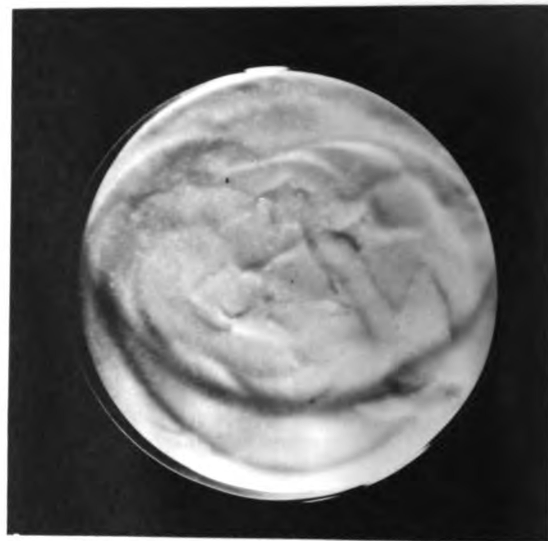
Results of the Panel Evaluation

Panel scores are summarized in Table III. The descriptive terms most frequently used in describing the various sauces may be summarized as follows:

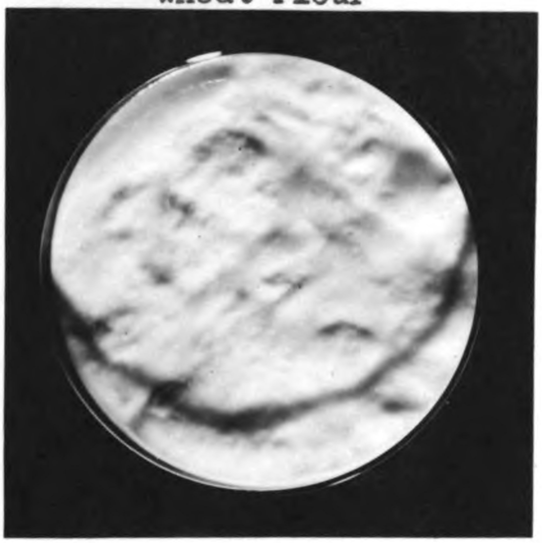
Control - homogeneous, no separation, velvety, bland, short, acceptable

Wheat flour sauces - large lumps, much separation, watery liquid, sandy, bland, friable, not acceptable

Wheat Flour



Unfrozen Wheat Flour



Waxy Rice Starch

Waxy Rice Flour

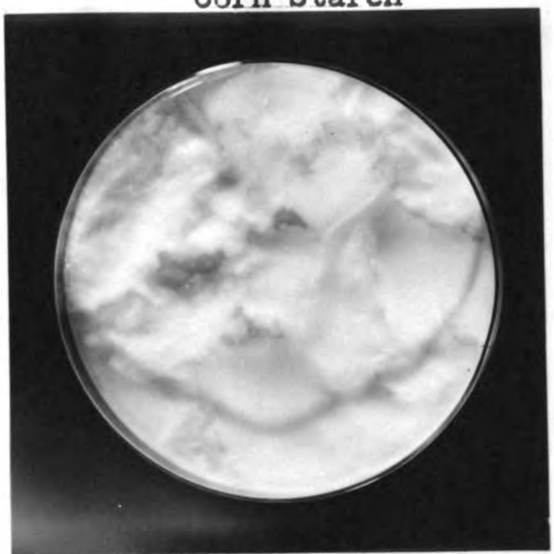
Cross-linked Waxy
Corn Starch

Figure 2. Appearance of sauces prepared from several thickening agents upon thawing at 35°C. after one month storage at -19.5°C.

Sorghum Starch

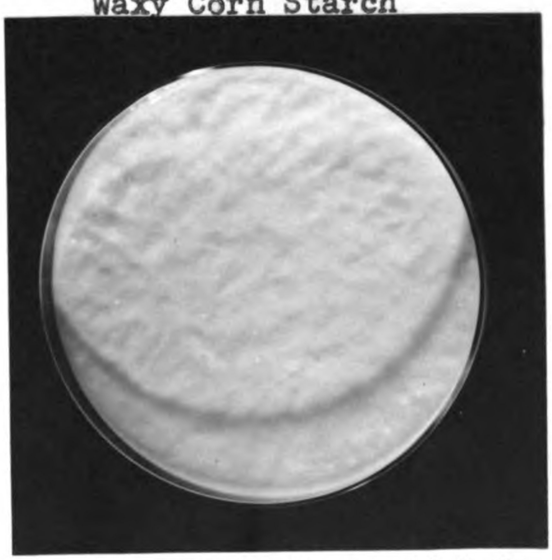
Corn Starch

Waxy Sorghum Starch



Waxy Corn Starch

Phosphate Cross-linked
Waxy Sorghum Starch



Phosphate Cross-linked
Waxy Corn Starch

Waxy rice flour sauces - homogeneous, no separation, velvety, short, bland, acceptable

Waxy rice starch sauces:
- 1 week: homogeneous, no separation, milky liquid, slippery, bland, not acceptable
- later: large lumps, much separation, watery liquid, slippery, off-flavor, not acceptable

Waxy corn starch sauces - large lumps, much separation, watery liquid, slippery, bland, stringy or gummy, not acceptable

Waxy sorghum starch sauces - large lumps, much separation, watery liquid, slippery, bland, stringy or gummy, not acceptable

Sorghum starch sauces - large lumps, much separation, watery liquid, sandy, bland, friable, not acceptable

Corn starch sauces - large lumps, much separation, watery liquid, sandy, bland, friable, not acceptable

Cross-linked waxy corn starch sauces - large lumps, some to much separation, watery liquid, sandy, raw cereal and off-flavor, short, not acceptable

Phosphate cross-linked waxy corn starch sauces:
- 1 week: large lumps, some separation, milky liquid, raw cereal and off-flavor, short, not acceptable
- later: large lumps, much separation, watery liquid, raw cereal and off-flavor, short, not acceptable

Phosphate cross-linked waxy sorghum starch sauces:
- 1 week: homogeneous, no separation, sandy, raw cereal and off-flavor, friable, gummy, acceptable
- later: large lumps, much separation, watery liquid, sandy, raw cereal and off-flavor, friable, gummy, not acceptable

Analysis of variance of the panel scores showed no significant differences in replications for any of the seven factors evaluated. Thickening agents showed very significant

differences in all factors except flavor, in which differences were significant. Storage times showed very significant differences in smoothness, separation, separated liquid, and acceptance, significant differences in mouth feel and paste character, and no differences in flavor. There was a very significant difference between scores of different judges on all factors except flavor in which there were no differences. It should be noted that each judge was relatively consistent in scoring and probably statistical differences were due to the range in scores of the panel members, for certain judges tended to give scores consistently higher than those of other judges. The statistical significance of various factors is summarized in Table IV. The details of the analysis may be found in the Appendix, Tables VIII through XIV.

Results of Objective Tests

The results summarized in Table V show the behavior of sauces and pastes prepared with the various thickening agents when subjected to centrifugation and other physical and chemical tests after frozen storage. In most cases, the initial change (after a one-week storage period) was great, and further storage caused only a slow continuation of the deterioration. However, with several thickening agents, notably waxy rice starch, waxy rice flour, phosphate cross-linked waxy corn starch, and phosphate cross-linked

TABLE IV

SUMMARY OF STATISTICAL ANALYSES

Factor	Thickening Agents	Storage Times	Judges	Replications
Smoothness	**	**	**	/
Separation	**	**	**	/
Separated Liquid	**	**	**	/
Mouth Feel	**	*	**	/
Flavor	*	/	/	/
Paste Character	**	*	**	/
Acceptability	**	**	**	/

* significant at 5% level   / Not significant

** significant at 1% level

See the Appendix, Tables VIII through XIV for details of the analysis.

waxy sorghum starch, the change during the first week of storage was small but an increase in storage time caused relatively large changes in the properties of the sauces.

Separated liquid in waxy rice flour sauces and pastes was not watery but was viscous and was difficultly separable from the sediment. The solid phase from sorghum starch and corn starch sauces and pastes tended to entrain liquid, making accurate, reproducible measurements of the volume of the liquid difficult. There was wide variation in turbidities of supernatant liquids with certain thickening agents, primarily in the group of derivatized starches.

A comparison of results obtained for samples stored six months and thawed at 35°C. and those thawed at 100°C. is possible from Table V. The contents of cans subjected to thawing at 100°C. reached temperatures of approximately 90°C. With the elevated thawing temperature there were visible differences as compared to samples thawed at 35°C. The waxy starch sauces - corn, sorghum, and rice - showed some visible watery liquid separation and had large, soft, slippery lumps in samples thawed at 100° C. These same starches produced sauces with considerably more watery liquid separation when thawed at 35°C. Corn starch and sorghum starch sauces showed considerable visible separation of a watery fluid and had a grainy appearance with many small lumps. This latter appearance is in contrast to that of similar sauces thawed at 35°C., which consisted of a single spongy mass with exuded water surrounding it. Wheat flour sauces appeared to be very grainy and had visible separated liquid if thawed at 100°C., while samples thawed at 35° C. were very lumpy with more liquid visible. Waxy rice flour sauces thawed at 100°C. appeared to be smooth and showed no visible separation. Those thawed at 35°C. showed a slight amount of separation but were smooth. The three derivatized starches gave sauces with soft lumps but no evidence of watery liquid separation was visible in samples thawed at 100°C. This is in sharp contrast to samples thawed at 35°C., which showed graininess and visible watery liquid separation.

The nitrogen content of supernatant liquids corresponded to turbidity readings of the corresponding samples which were stored one month.

Observations of Sauces and Pastes Prepared with Glycogen and Non-Granular Amylopectins before Freezing

The tapioca branched fraction gave a stringy paste when mixed with water. However, on heating, the stringiness disappeared below 80°C. The sago fraction was more difficult to disperse in water, complete dispersion being effected only by heating to 92°C. The waxy maize fraction formed a tacky mass with water, which was somewhat dispersed on heating to 95°C., but at this temperature some

undispersed starch remained and dispersion occurred on heating an additional 15 minutes. Glycogen dispersed readily and the dispersion cleared somewhat at 85° C.

The sauces prepared with glycogen and the sago starch fraction did not emulsify fat, as well as those prepared with tapioca and waxy maize fractions. Glycogen and the sago fraction showed less apparent thickening than did tapioca and waxy maize fractions. All produced much less thickening than did the granular starches. The thickening agents used as checks behaved as noted previously.

Unfrozen samples standing at room temperature for two days showed no precipitate. The pastes from the sago and maize amylopectins and water, after storage for two days in the freezer, were thawed, giving lumpy products. The thawed paste from the sago fraction was heated to 60°C., whereupon the lumps disappeared and did not reform after standing at room temperature for two days. The thawed paste from the waxy maize fraction was allowed to stand at room temperature for two days without further heating; no apparent change in clarity occurred.

Results of Objective Tests on Glycogen and Nongranular Amylopectin Samples after Freezing and Subsequent Thawing

The appearances of sauces and pastes prepared with nongranular amylopectins and glycogen, as well as certain

TABLE VI

MACROSCOPIC APPEARANCE OF THAWED SAUCES AND PASTES PREPARED WITH SEVERAL THICKENING AGENTS AFTER ONE WEEK STORAGE AT -19.5°C.

Thickening Agent	Water Pastes	Sauces
Corn Starch	Much lumping & watery liquid separated	Very lumpy & with much watery liquid separated
Phosphate Cross-linked Waxy Corn Starch	Some lumping	Very flocculent Slight separation
Waxy Corn Starch	Marked lumping	Several large lumps Considerable separation
Waxy Rice Starch	Some separation	Some lumping
Waxy Rice Flour	Slight separation	No separation
Tapioca Starch Amylopectin	No lumping	No lumping
Waxy Maize Branched Material	Slight lumping	Slight lumping
Sago Starch Amylopectin	Very noticeable lumping	Very noticeable lumping
Glycogen	No lumping	No lumping

thickening agents used as controls, thawed at 27°C. after a one-week storage period are given in Table VI. The control samples appeared as they did in previous observations. There were, however, marked differences in appearances of the other samples.

The results of objective evaluation of these samples are summarized in Table VII. The samples which were included as checks behaved in the same manner as in previous tests. In samples stored three months, the results were more difficult to explain than in samples stored one week. Skimmed milk which was subjected to the experimental conditions showed some instability after the dry-ice packing in transfer and the glycogen and tapioca fraction sauces gave results comparable to the values obtained with milk. However, glycogen and tapioca amylopectin pastes showed no separated material even after the dry-ice shipping.

DISCUSSION

On the basis of the results presented, it is evident that the rate and extent of lumping induced by frozen storage of cooked starch-containing mixtures vary greatly, according to the type of starch used. Lumping was apparent after freezing systems containing only starch or flour and water and was not necessarily dependent on the presence of milk. Correlation between lumping of starch-water pastes and white sauces prepared from the corresponding starches indicated that starch was the primary factor in causing the behavior. Marked differences in the turbidity of the separated liquids were observed. Kjeldahl analysis of the liquids, separated by centrifugation, showed a correlation between nitrogen content and turbidity. Use of starches such as corn starch and sorghum starch and wheat flour which contain relatively high amounts of straight chain fraction (amylose) resulted in the separation of liquids which had a low protein content. The waxy starches, which contain primarily amylopectin, were in an intermediate group while the derivatized waxy starches showed relatively large amounts of nitrogenous material in the supernatants following a one-month period of frozen storage.

When this information is considered in conjunction with the observations of the lumps formed, it appears that there may be formed a network of starch molecules (either those released from broken granules or those at the granule surface) which entrains and/or binds the protein molecules into the interior of the aggregate, thus effectively removing nitrogenous material from the liquid. Since the size of such aggregates would be quite large, the aggregate is no longer dispersable and is precipitated. The fact that the cross-linked starches produce fewer chains free for the formation of such a network may explain their smaller tendency to form such lumps, especially in the early storage period. In such instances, combination between free side chains is greatly lessened since the surface chains are partially linked together by a phosphate (or other) bridge and are not available for starch to starch interaction. The cross-linking is not entirely satisfactory in preventing lumping since such sauces are not free from obvious lumping, especially with longer storage periods. Possibly this continued action during storage is due to an opportunity for further orientation of the "sandy" particles formed early in storage such that opportunity for interaction of the starch is possible. Cross-linking does not prevent aggregation and lumping, but merely decreases the rate of aggregation and size of the resulting lumps.

The behavior of a highly ramified structure as found in glycogen is interesting in this respect. The outer branches of glycogen are relatively short and offer less chance of intermolecular attraction than do the outer branches of the waxy starches which were studied. The outer branches of amylopectins are approximately 15 to 18 glucose units in length while the outer branches of glycogen are 6 to 7 glucose units in length, according to Meyer (22). Even though glycogen, being nongranular, is exposed to the system on all sides, aggregation does not occur. From the behavior of the nongranular amylopectins it is evident that possession of granular structure is not requisite to aggregation. The lumps formed in nongranular amylopectin sauces tended to be smaller than those in granular waxy starch sauces. Possibly this difference is due to the absence of the granules. Thus whole granules are not involved when intermolecular binding occurs, the result being a smaller aggregate with the nongranular starches.

The widely varied behavior of nongranular amylopectins obtained from several starches cannot be related to either average branch length or average molecular weight, since in two of the fractions studied (tapioca and sago amylopectins) which gave sauces with a great difference in stability, Potter and Hassid (40, 41) found these values to be nearly the same. The waxy rice starch gave nearly the same value for average branch length when the same method of analysis was used (13).

It is apparent from the observations and data presented that certain fine details of structure of the amylopectins must vary considerably. Although average branch length of the several amylopectins was the same, the behavior of these amylopectins varied greatly with respect to stability when pastes or sauces were frozen. It is possible that two amylopectins with approximately equal average branch lengths would vary greatly with respect to distribution of branch length. An amylopectin with branches of relatively uniform branch length could have the same average branch length as an amylopectin in which there were very long and very short branches as well as some in the intermediate range. The same situations can be postulated for the molecular weight distribution.

That retrogradation of starch is a primary factor in the instability of frozen sauces was further supported by the results obtained when the sauces were thawed in boiling water. Since this treatment raised the temperature of the sauces to approximately 90°C., the retrograded amylopectin portion would be expected to redisperse (47). The appearance of sauces treated in this manner was considerably more desirable (smoother with less visible liquid separated) than corresponding sauces stored the same period of time but heated to 35°C. Also the sauces thawed in boiling water gave less separated liquid by centrifugation. Turbidities of these liquids were also greater. The behavior of the

waxy starches is especially striking in these respects: separated liquid was reduced to about 20% to 50% of that obtained with sauces thawed at 35°C. The cross-linked starches also showed a reduction in liquid separated by centrifugation. The ordinary starches were improved less than the others by the boiling water thaw. The relatively high amylose content of these starches is undoubtedly responsible for this behavior since retrograded amylose is highly insoluble and heating to 90°C. under the prevailing conditions would not effect dispersion (47).

Of the samples heated to 90°C. after a six-month frozen storage period, only waxy rice flour sauces were free from visible liquid separation and lumps. A frozen food product consisting of a white sauce base would be expected to behave much like the sauces alone do, especially if thawed without agitation (as in meat pies, frozen oven dinners, or items thawed over boiling water). The information obtained in this study suggests that, of the thickening agents used which are available in commercial quantities, waxy rice flour is the only one showing sufficient stability to freezing to give a product which is acceptable if heated to 90°C. in thawing. Stability even with this thickening agent was not sufficient if the product was not subjected to temperatures above 35°C. in thawing. Waxy rice starch does not show this unusual stability, even when thawed at 100°C. Since the starch was isolated from a portion of waxy rice flour from the same lot as the flour used in the study, there seems to be a stabilizing factor in the flour. This stabilizing factor has not been defined or investigated in this study.

Sauces prepared with the amylopectin from tapioca starch showed unusual stability, even when thawed at 27°C. These samples appeared to be stable even under conditions which caused destabilization of the milk. This behavior seems explainable only on the basis of the molecular structure of the tapioca amylopectin.

SUMMARY

The behavior of ten different thickening agents was studied with respect to stability of frozen white sauces. Wheat flour, corn starch, sorghum starch, waxy corn starch, waxy sorghum starch, waxy rice starch, waxy rice flour, a cross-linked waxy corn starch, a phosphate cross-linked waxy corn starch, and a phosphate cross-linked waxy sorghum starch were used in amounts which, together with a given amount of skimmed milk, of sodium chloride, and of hydrogenated vegetable oil, gave a final viscosity of about 30 gram-centimeters as measured five minutes after maximum viscosity or ten minutes after the start in the increase in viscosity (in samples giving no maximum) when cooked in the Corn Industries Viscometer. Samples were frozen in tin cans and held at -19.5°C. for varying periods of time.

A taste panel found all sauces except that prepared with waxy rice flour unacceptable after a one-month storage period. The panel scores indicated that the changes were great with a one-week storage period and longer storage only accentuated differences.

The panel scores coincided with centrifugation and turbidity tests on liquid separated from the frozen sauces upon thawing. Differences in turbidity paralleled protein

content of the supernatant.

From results obtained on sauces thawed at 100°C., it appeared that retrogradation of starch was the primary factor in instability.

Sauces prepared from nongranular amylopectins showed widely varying behavior and stability which was not related to average branch length or average molecular weight. Sauces and pastes prepared from glycogen and tapioca branched fractions were stable to freezing while waxy maize amylopectin and sago starch amylopectin gave unstable sauces and pastes when thawed at 27°C. after a one-week storage period.

CONCLUSIONS

White sauces prepared with wheat flour, corn starch, sorghum starch, waxy corn starch, waxy sorghum starch, waxy rice starch, phosphate cross-linked waxy corn starch, phosphate cross-linked waxy sorghum starch, and cross-linked waxy corn starch showed marked instability within a one-week period when stored frozen at $-19.5^{\circ}C.$ and thawed at $35^{\circ}C.$ Sauces prepared from waxy rice flour showed instability only after a three-month storage period. The instability appears to be related to a retrogradation of the starch components as well as to the secondary destabilization of milk proteins. Storage times and thickening agents were statistically significant with respect to their influence on several factors evaluated. Centrifugation of sauces and turbidity tests of supernatant liquor further supported evidence of a progressive destabilization. Protein content of the supernatant was related to the turbidity measurements, indicating either physical or chemical involvement of protein in the retrograded starch network. The nature of this involvement was not determined in this study. Since instability of starch-water pastes paralleled that of the frozen sauces, it appeared that the protein was not a major factor in the separation of the mixtures.

Studies on behavior of nongranular amylopectins showed that granular structure neither prevented nor caused the instability. Instability could not be related to either average branch length or average molecular weight. Certain details of the fine structure of amylopectins are not known and methods for their determination are at present inadequate. The need for further studies on the structure of amylopectins is indicated by the results of this project.

APPENDIX

APPENDIX

TABLE VIII

ANALYSIS OF VARIANCE OF PANEL SCORES FOR SMOOTHNESS

Source of Variation	S.S.	D.F.	M.S.	F Value
Total	1894.52	599		
Thickening Agents	861.97	9	95.78	7.97[a]**
Storage Times	242.50	2	121.25	10.13[a]**
Judges	131.04	4	32.76	9.75[a]**
Replications	5.46	3	1.82	0.22[a]
Thickening Agents x Storage Times	135.30	18	7.52	11.13**
Thickening Agents x Judges	120.92	36	3.36	4.97**
Thickening Agents x Replications	30.56	27	1.14	1.68*
Storage Times x Judges	7.95	8	0.99	1.47
Storage Times x Replications	26.74	6	4.46	6.60**
Judges x Replications	11.92	12	0.99	1.47
Error	320.17	474	0.68	

[a] computed using pooled interaction error
* significant at 5% level
** significant at 1% level

TABLE IX

ANALYSIS OF VARIANCE OF PANEL SCORES FOR SEPARATION

Source of Variation	S.S.	D.F.	M.S.	F Value
Total	2589.96	599		
Thickening Agents	1445.77	9	160.64	11.32[a]**
Storage Times	388.42	2	194.21	7.58[a]**
Judges	73.88	4	18.47	5.31[a]**
Replications	3.83	3	1.28	0.05[a]
Thickening Agents x Storage Times	214.51	18	11.92	20.77**
Thickening Agents x Judges	81.72	36	2.27	3.96**
Thickening Agents x Replications	17.55	27	0.65	1.13
Storage Times x Judges	9.68	8	1.21	2.11*
Storage Times x Replications	75.00	6	12.50	21.78**
Judges x Replications	7.54	12	0.63	1.10
Error	272.04	474	0.57	

[a] computed using pooled interaction error
* significant at 5% level
**significant at 1% level

TABLE X

ANALYSIS OF VARIANCE OF PANEL SCORES FOR SEPARATED LIQUID

Source of Variation	S.S.	D.F.	M.S.	F Value
Total	2571.76	599		
Thickening Agents	1397.56	9	155.28	10.78[a]**
Storage Times	416.19	2	208.10	8.00[a]**
Judges	57.36	4	14.34	4.29[a]**
Replications	4.73	3	1.58	0.12[a]
Thickening Agents x Storage Times	223.01	18	12.39	20.27**
Thickening Agents x Judges	72.17	36	2.00	3.28**
Thickening Agents x Replications	16.67	27	0.62	1.00
Storage Times x Judges	10.66	8	1.33	2.18*
Storage Times x Replications	73.92	6	12.32	20.16**
Judges x Replications	9.80	12	0.82	1.34
Error	289.69	474	0.61	

[a] computed using pooled interaction error
* significant at 5% level
**significant at 1% level

TABLE XI

ANALYSIS OF VARIANCE OF PANEL SCORES FOR MOUTH FEEL

Source of Variation	S.S.	D.F.	M.S.	F Value
Total	1768.78	599		
Thickening Agents	718.27	9	79.81	6.48[a]**
Storage Times	117.73	2	58.86	5.55[a]*
Judges	240.16	4	60.04	9.98[a]**
Replications	1.46	3	0.49	0.11[a]
Thickening Agents x Storage Times	146.34	18	8.13	11.89**
Thickening Agents x Judges	150.61	36	4.18	6.12**
Thickening Agents x Replications	23.73	27	0.88	1.29
Storage Times x Judges	9.57	8	1.20	1.75
Storage Times x Replications	14.86	6	2.48	3.62**
Judges x Replications	22.00	12	1.83	2.68**
Error	324.07	474	0.68	

[a] computed using pooled interaction error
* significant at 5% level
**significant at 1% level

TABLE XII

ANALYSIS OF VARIANCE OF PANEL SCORES FOR FLAVOR

Source of Variation	S.S.	D.F.	M.S.	F Value
Total	1466.39	599		-
Thickening Agents	208.83	9	23.20	2.19[a]*
Storage Times	40.25	2	20.13	1.72[a]
Judges	157.69	4	39.42	2.32[a]
Replications	22.51	3	7.50	1.02[a]
Thickening Agents x Storage Times	62.61	18	3.48	2.85**
Thickening Agents x Judges	256.71	36	7.13	5.85**
Thickening Agents x Replications	25.45	27	0.94	0.77
Storage Times x Judges	43.00	8	5.37	4.41**
Storage Times x Replications	17.27	6	2.88	2.36*
Judges x Replications	53.85	12	4.49	3.68**
Error	578.22	474	1.22	

[a] computed using pooled interaction error
* significant at 5% level
**significant at 1% level

TABLE XIII

ANALYSIS OF VARIANCE OF PANEL SCORES FOR PASTE CHARACTER

Source of Variation	S.S.	D.F.	M.S.	F Value
Total	1852.40	599		
Thickening Agents	852.11	9	94.68	6.49[a]**
Storage Times	143.50	2	71.75	5.59[a]*
Judges	111.82	4	27.96	4.11[a]**
Replications	1.40	3	0.47	0.05[a]
Thickening Agents x Storage Times	170.83	18	9.49	13.33**
Thickening Agents x Judges	129.04	36	3.58	5.04**
Thickening Agents x Replications	40.34	27	1.49	2.10**
Storage Times x Judges	7.50	8	0.94	1.32
Storage Times x Replications	20.11	6	3.35	4.71**
Judges x Replications	38.27	12	3.19	4.48**
Error	337.46	474	0.71	

[a] computed using pooled interaction error
* significant at 5% level
**significant at 1% level

TABLE XIV

ANALYSIS OF VARIANCE OF PANEL SCORES FOR GENERAL ACCEPTABILITY

Source of Variation	S.S.	D.F.	M.S.	F Value
Total	1555.98	599		
Thickening Agents	594.24	9	66.03	6.54[a]**
Storage Times	152.53	2	76.26	5.97[a]**
Judges	228.24	4	57.06	9.36[a]**
Replications	3.28	3	1.10	0.18[a]
Thickening Agents x Storage Times	129.07	18	7.17	12.56**
Thickening Agents x Judges	105.26	36	2.92	5.12**
Thickening Agents x Replications	16.33	27	0.60	1.06
Storage Times x Judges	11.34	8	1.42	2.48*
Storage Times x Replications	25.11	6	4.18	7.33**
Judges x Replications	19.89	12	1.66	2.90**
Error	270.70	474	0.57	

[a] computed using pooled interaction error
* significant at 5% level
**significant at 1% level

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