THE EFFECT OF SUCROSE AND CALCIUM CYCLAMATE UPON THE GEL STRENGTH OF SELECTED GELLING AGENTS IN THE PREPARATION OF JELLIED CUSTARD

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Mary Ellen Zabik

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ABSTRACT

THE EFFECT OF SUCROSE AND CALCIUM CYCLAMATE
UPON THE GEL STRENGTH OF SELECTED GELLING AGENTS
IN THE PREPARATION OF JELLIED CUSTARD

by Mary Ellen Zabik

The purpose of this study was to compare the effects of sucrose and calcium cyclamate upon the gel strength of jellied custards prepared with gelatin and two marine hydrocolloids, carrageenan and algin.

The basic formula consisted of constant proportions of all ingredients except the gelling and sweetening agents. Representatives of the gelling agents, chosen arbitrarily from products recommended for use in milk-type dessert gels, were pure, unflavored, acid-processed gelatin with a bloom value of 220; carrageenan;[1] and algin preparation[2] which were added in the predetermined gram-ratio of 1.00 : 0.179 : 0.857, respectively. Custards gelled with each agent were sweetened by the following 5 methods: with 20 and 40 per cent sucrose, based on total formula weight exclusive of water; with concentrations of dry calcium cyclamate equivalent in sweetness to the sucrose concentrations selected; and without sweetener for a control.

The mean batch yield for custards increased significantly with the increased weight of sweetener. Since differences in total weight of

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[1]Type 10 Carrageenan manufactured by Marine Colloids, Inc., Chicago 5, Ill.

[2]Margel, a mixture of algin and calcium carbonate, manufactured by Kelco Company, Chicago 6, Ill.

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ingredients affect dilution of the gelling agent, it is conceivable that gel strength differences apparent in this study may be due, in part, to variation in batch yield.

Significant differences for gel strength were established from penetration and per cent sag data. Custards gelled with algin preparation or gelatin exhibited greater gel strength than custards gelled with carrageenan. The rank order for gelatin and algin preparation custards varied with the type and concentration of sweetener present. Sucrose-sweetened custards gelled with algin preparation seemed to have greater gel strength than sucrose-sweetened custards gelled with gelatin whereas an inverse gel strength relationship occurred for cyclamate-sweetened samples gelled with these two agents.

Per cent sag data showed rigidity of carrageenan gels decreased with sucrose addition and this effect was greater as the concentration of sucrose increased. Penetration data, however, did not support these findings.

The addition of dry calcium cyclamate seemed to increase gel strength for gelatin custards and to decrease gel strength for both carrageenan and algin preparation custards. This effect was increased as the concentration of calcium cyclamate was increased. Custards gelled with algin preparation and sweetened with the higher concentration of calcium cyclamate exhibited the largest decrease in rigidity.

Variance in the weight of drainage due to syneresis among treatments was found to be significant. Syneresis was evident only for custards gelled with carrageenan and drainage appeared to be enhanced by the addition of dry calcium cyclamate.

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The findings of this limited study indicate custard gel strength is a function of gelling agent and type and concentration of sweetener used. Suggested areas for supplementary study include equilibrating the amount of gelling agent used with total weight of ingredients and/or substituting sodium cyclamate for calcium cyclamate. Further investigations are warranted to establish whether syneresis is inherent in the milk-type carrageenan gel or whether formula modification can overcome this drainage.

THE EFFECT OF SUCROSE AND CALCIUM CYCLAMATE UPON THE GEL STRENGTH OF SELECTED GELLING AGENTS IN THE PREPARATION OF JELLIED CUSTARD

By

Mary Ellen Zabik

A THESIS

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INTRODUCTION

The curtailed availability of agar and gum arabic from European sources during World War II resulted in the development of polysaccharides from seaweeds as effective industrial substitutes. Recent advances in the application of these marine hydrocolloids, particularly, the carrageenates and the alginates, to the manufacture of industrial products requiring controlled gelation and/or stabilization have stimulated interest in the feasibility of their use as gelling agents and stabilizers in food preparation.

Carrageenan[1] is extracted from certain salt water plants, variously called Irish Moss or carrageen (rock) moss, which are designated more exactly as Chrondus crispus or Gigartina stellata in the class Rhodophyceae or red algae, whereas alginic acid is extracted from a number of different species and genera all of which belong to the class Phaeophyceae or brown algae. Both the Eastern and Western coastal waters of the United States serve as readily available sources of the brown and red algae. However, the brown algae grows prolifically in polar and temperate climates while it shuns tropical regions; therefore the principal American sources of the alginates are the giant kelp, Macrocystis pyrifera, located in the Pacific coastal waters from Alaska to California and the Laminaria digitata found in the North Atlantic

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[1]Carrageenan is the recommended nomenclature of the American Chemical Society. It is also referred to as carrageenin, carragenin, carrageen, carragheen, carragheenin, Irish Moss, and Irish Moss Extractive.

Ocean, particularly in the coastal waters of Nova Scotia. In contrast to this, the red algae flourishes in more temperate climates; thus the coastal waters of Florida and California are the principal American sources of carrageenan, which in the United States is obtained almost entirely from Chrondus crispus.

Although the gelation and/or stabilization properties of the carrageenates and the alginates have been established, the literature contains little basic information relative to the gelling efficiency of these products in contrast with agents, such as cornstarch and gelatin, which are more commonly used in food preparation.

The effect of sucrose upon the gel strength of gelatin desserts has been firmly established. Little is known about the effect of artificial sweeteners, such as calcium cyclamate and sodium cyclamate, upon the functional efficiency of gelatin in such products. The effect of sucrose and/or artificial sweeteners upon the gel strength of desserts prepared with marine hydrocolloids has not been reported in the literature.

If marine hydrocolloids can be used successfully in food preparation, they will be potential additions to the types of gelling agents and/or stabilizers currently available to the food service operator. Moreover, if it is possible to substitute artificial sweeteners for sucrose in the preparation of molded desserts made with gelatin, carrageenates, and/or alginates without sacrificing the quality of these products, such menu items could increase the variety of desserts available for diets with sucrose restrictions.

The purpose of this investigation is to compare the effect of two concentrations of sucrose and two concentrations of calcium cyclamate

(comparable in sweetness to the concentrations of sucrose tested) upon the gel strength of custards made with pure gelatin, carrageenan, and alginic acid. The gel strength data will be examined to determine (a) the practicability of using carrageenates and/or alginates as gelling agents in the preparation of jellied milk-type desserts and (b) the possibility of substituting calcium cyclamate for sucrose in the preparation of jellied milk-type desserts made with pure gelatin, carrageenan, and alginic acid.

REVIEW OF LITERATURE

The gelation process and the gel structures formed in heterogeneous mixtures such as organic gels are complex. Three theories of structural formation, advanced by various investigators and summarized by Weiser (72), are: (a) gels are composed of crystalline threads, (b) gels are made up of a framework of amorphous threads, and (c) gels are not composed of either crystalline or amorphous threads but are irregular groupings of particles. Each theory is probably correct in specific cases. Furthermore, it is possible various arrangements of molecular aggregates occur simultaneously in the same gel. In all cases it seems probable that the particles are highly hydrous as a result of adsorption or absorption and that they are linked together, forming an irregular mesh or network which entrains liquid in the interstices.

Practically all of the organic gelling agents swell in the presence of suitable liquids or vapors and, with proper temperatures, will peptize to form sols which are influenced by pH and presence of salts. Elastic gels formed from these agents lose water continuously in dry air; moreover with proper temperatures and humidity hydration will occur. These gels exhibit aging, which may manifest itself by agglomeration of colloidal particles to form denser aggregates which can absorb and entrain less liquid than the freshly prepared system. The aged system exudes liquid, a phenomenon called syneresis. Syneresis appears to be influenced by setting time; the quicker the gel sets the sooner syneresis occurs and the more rapid the initial velocity of the process. In addition, each organic gelling agent is affected by its own source and method of manufacture as well as characteristics peculiar to that agent alone.

Gelatin

Gelatin is the collective term for an extremely heterogeneous protein composed of many-sized polypeptides. Gelatin's main distinction rests in its ability to form gels at relatively low concentrations. Anding (6) describes "pure" dry gelatin as a tasteless, odorless, transparent, brittle, glasslike solid, very faint yellow to amber in color. Although gelatin never occurs in nature, it is derived from the animal constituent collagen by an irreversible hydrolytic process. The gelling efficiency of any gelatin sample is a function of the collagen source, method of manufacture, thermal history, pH, presence of electrolytes, presence of non-electrolytes, concentration, and molecular weight.

Collagen source

Collagen, the principal intercellular constituent of the white connective tissue of animal skins and bones, is the essential raw material of gelatin. Collagen is procured from hides obtained in the United States and imported from South America and England and from bones imported from South America and India. Pigskins as well as cattle hides are used extensively in the United States whereas the production of gelatin from ossein, the organic material left after the removal of minerals from bone, is less. In contrast to this, Europe produces 20 to 30 per cent more gelatin from ossein than from hides.

Manufacturing procedures

Modern procedures in gelatin manufacture are all designed to render collagen more readily convertible to gelatin. This is accomplished by previous chemical treatment, either alkaline or acid processing, which

reduces the time gelatin is exposed to high temperatures and thereby reduces degradation of the product.

Alkaline process (36). For this method the raw materials are soaked in a calcium hydroxide suspension for 1 to 6 months at pH 12 to 12.5 at temperature of the surrounding air. The total amount of lime used is approximately 10 per cent of the weight of the stock although considerably less is required if either sodium hydroxide or sodium carbonate is also used. High concentrations of soluble alkali are disadvantageous due to their corrosive action on skin causing a loss of material while inner portions remain unaltered. Nevertheless, concentrations of approximately 1 per cent are often used to "sharpen up" lime liquors. Liming changes collagen into a form which is more easily converted to gelatin as well as removes a number of impurities insuring gelatins of better color and clarity. After liming is finished, the materials are washed with clear water and/or dilute acid to affect complete removal of the alkali before extraction. Acid hastens the process, increases swelling, and removes salts.

In a laboratory experiment on dried sinew, Ames (4) found both yield and gel strength increase with increased time of liming and with approximately 3 months of liming a gelatin with a bloom value of 250 could be produced. In previous experiments Ames (5) attempted to shorten the lime soak by "sharpening" the liquor. He reported that at temperatures ranging from 16 to 20°C, the maximum safe concentrations of sodium hydroxide and sodium carbonate which could be used along with the lime soak were 0.5 and 0.7 per cent, respectively. Increasing the temperature of the soak above 22 to 25°C hastened the swelling time but

produced an inferior product and caused destruction of the stock. Ames also stated that soluble alkali could not be used along with the lime soak at increased temperatures.

Acid process (36). Acid processing is used primarily for frozen pigskins in the United States and for ossein in Europe. Pigskins, previously frozen into molds of approximately 100 pounds, are thawed with water washes, soaked in dilute solutions of hydrochloric, sulfuric, or phosphoric acid until penetrated, and washed with fresh water until sufficient acid is removed to elevate the pH to 4, the desired acidity for cooking. At this pH impurities are relatively insoluble and remain as residue at the end of extraction.

Extraction, filtration, drying. Gelatin from either limed or acidified stock is extracted with successive portions of water increasing in temperature from 55 to 100°C. Anding (6) reported the following extraction data: first extraction at 55 to 65°C for 4 to 9 hours yields 5 to 10 per cent gelatin, second extraction at 65 to 75°C for 4 to 8 hours yields 3 to 6 per cent gelatin, third extraction at 75 to 85°C for 4 to 6 hours yields 3 to 6 per cent gelatin, fourth extraction at 85 to 95°C for 4 to 6 hours yields 2 to 4 per cent gelatin, and the final extraction at 95 to 100°C for 2 to 4 hours yields 1 to 2 per cent gelatin. Idson and Braswell (36) have stated the increasing temperature of each successive cook causes the gel-forming power to drop. These gelatin liquors are filtered through pressure filters and the filtrate is placed in a multiple-effect evaporator where 50 to 75 per cent of water is removed. The initial stage of drying, accomplished with a 32°C dry-bulb,

reduces the moisture content to 20 to 30 per cent. This step is followed by humidity-controlled hot drying at 32 to 60°C which reduces the moisture to approximately 10 per cent. Finally the dried residue is treated mechanically to produce the desired physical form: sheet, flake, or granule.

Composition

Gelatin is composed of a minimum of eighteen different amino acids linked together in a partially ordered fashion. Numerous investigators have established an accurate amino acid pattern for gelatin. Idson and Braswell (36) reported the composition of the gelatin molecule as approximately 1/3 glycine, 1/3 cyclic amino acids such as proline and hydroxyproline, 1/5 dibasic or diacidic amino acids, and small amounts of a variety of other amino acids. Functional groups of gelatin reported by Ward (71) are the amino, imidazole, guanidino, carboxyl, hydroxyl, and peptide bond groups. The first three carry a positive charge, the carboxyl carries a negative charge, and the last two carry no charge. Although the composition of gelatins from alkaline or acid processes is similar, Ward reported more carboxyl groups in alkaline- than in acid-processed gelatin.

Gel formation

Gelation is the formation of a gel or the solidification of a sol. Lowe (39) pointed out that one of the outstanding characteristics of gelatin is its ability to form a sol at 35°C or higher and, with high enough concentration, a gel at lower temperatures. This sol-gel transformation is thermally reversible. Accompanying gelation the following

changes occur in the gelatin system: changes in viscosity, rigidity, elasticity, optical rotation, surface films, electrical conductivity, and X-ray diagrams. Ferry (22) stated rigidity, a characteristic common to all gels, appears suddenly at the moment of gelation and the changes in optical rotation which occur with time follow very closely rigidity changes. Richardson (54) proposed that the acquirement of elasticity distinguishes the gel from a suspension. Viscosity of sol and rigidity of gel may run parallel, but this is not always true for gelatins giving the most viscous sols do not always yield the stiffest gels.

Ferry (22) correlated the general theories applying to the mechanism of gelation of gelatin and presented evidence that individual molecular chains are bound together by secondary attractive forces localized at widely separated points. This locus of attraction actually includes several amino acid residues. In contrast, at the isoelectric point non-localized forces are predominate if there is no salt present. From their studies of the change in optical activity accompanying gelation, Ferry and Eldridge (23) concluded the rigidity of a gel is due to intramolecular crosslinks or intramolecular rearrangement, while comparatively small contributions are made by intermolecular links.

Boedtker and Doty (11), from their studies of aggregate and gel formation in gelatin systems, proposed association occurs through crystallite formation. This thesis is supported by the dependency of aggregate size on thermal history of the sample, a characteristic of polycrystalline materials. These investigators reported ionic strength of the sol did not appreciably influence bond formation, concluded

electrostatic forces do not play a dominant role, and suggested the remaining possibilities of hydrogen bonds, dispersion forces, and dipole interaction do contribute.

Earlier Ferry (22) presented evidence for two kinds of Van der Waals forces, hydrogen bonds and nonpolar attractions, which are presumed to be important in gelation of proteins and suggested both of these might be involved in the gelation of gelatin. The exact location of the bonding forces is not known; however Morel and Grabar (44) believe the guanidine group is essential to the formation of gelatin gels. Oxidative destruction of 45 per cent of the arginine prevented gel formation whereas milder oxidation without destroying the arginine weakened but did not destroy the ability of the gelatin to form a gel. These co-workers also reported destruction of 20 per cent of arginine and 15 per cent of hydroxyproline destroyed the gel forming power. Thus, it is suggested the hydroxyl groups are secondarily involved. Idson and Braswell (36) indicated Gustavson supported the possible important role of hydrogen bonds of the keto-imide linkage and Kinchington disputed the role of arginine in the gelling process.

Gel strength

Idson and Braswell (36) stated when a stress is placed upon a gelatin gel for a short time, the gelatin gel gives by an amount proportional to the applied force. Resistance to stress is a function of rigidity or gel strength and is affected by the collagen source, method of manufacture, and previous thermal history of the gelatin sample. In addition to these, gel strength is also influenced by the presence of electrolytes and/or non-electrolytes, pH, time and temperature of setting, concentration, and molecular weight.

Presence of electrolytes. Gelation temperature is affected by the presence of electrolytes. Lowe (39) pointed out substances which lower the melting point of gelatin also decrease rigidity. Lowe stated the effect of anions at equal concentrations is usually given for above the isoelectric point as follows: sulfate > citrate > tartrate > acetate > chloride > chlorate > nitrate > bromide > iodide. Sulfate usually elevates the gelation temperature, while iodide may lower the gelation temperature below 0°C. Bello et al (9) found fluoride increased the melting point whereas salicylate, tribromoacetate, and diiodisalicylate prevented gelation. The lowering of melting point was explained as the result of materials breaking peptide hydrogen bonds which were involved in maintaining the intramolecular crosslinks. The effectiveness of certain large ions as melting point reducers may be due to their ability to cover the peptide groups. The anions which raise the melting point may do so by protecting ordered segments of gelatin from denaturation by water or by cross-linking through interaction at the peptide links.

Lowe (39) suggested cations may have more effects than anions below the isoelectric point. Nobel (48) found all alkali metal cations had the same effect on elasticity of gelatin gels except lithium which had a slightly greater effect. The range of alkaline earth cations is as follows: barium > strontium > calcium > cesium = magnesium. All of these had a greater effect than water alone. Dahlberg et al (18) reported greater gel strength in milk systems than in water and stated the presence of salts in milk may be one reason for altered gel strength.

Presence of non-electrolytes. Friedman and Shearer (25) studied the effect of sucrose, urea, and levulose upon the setting time of gelatin

solutions. Small concentrations of non-electrolytes increased setting time; this effect was maximum at 0.02 to 0.03 M. Gelatin gels set more rapidly at concentrations exceeding 0.1 M. than in the absence of non-electrolytes; however 0.2 M. was the highest concentration they used. These workers found diffusion velocity varied directly with time of setting and slower setting gels exhibited a more open structure. Advani and Narwani (1) stated aldo and keto sugars condense with amino acid groups of a gelatin sol. Although this phenomenon does not occur with non-reducing disaccharides, there is a decrease in the free solvent of the system due to hydration of sugar.

pH. Boedtker and Doty (11) found gels formed at the isoelectric point in the absence of salts are weak; nevertheless, if the pH is more than one unit away from the isoelectric point, weak gels are no longer formed. With sufficient salt present pH makes little difference. Idson and Braswell (36) cited the following data of Gerngross: for a 10 per cent gelatin solution rigidity was independent of pH between 4.4 and 9.0, for a 3 per cent solution rigidity was independent between 4.6 and 8.2, and for a 1.5 per cent solution the rigidity was independent of pH between 4.3 and 6.7.

Time, temperature. Lowe (39) indicated the slower a gelatin solution is cooled the higher the temperature of gelation; all gels become firmer at low temperatures than at high temperatures; and above 35°C no gel is formed at any concentration of gelatin. Moreover, after a gel has formed, its firmness increases with time of standing. Ferry (21) stated rigidity decreases rapidly with increasing temperature and vanishes at about 30°C.

Gels cooled at 15°C develop high rigidity whereas gels cooled at 0°C and then returned to 15°C take ten times as long to develop comparable rigidity. This phenomenon was further studied by Eldridge and Ferry (19). Their results show some crosslinks are more stable than others. Thus, when gelation is allowed to proceed slowly stable crosslinks are formed. However, when the solution is chilled rapidly, crosslinks are formed in a haphazard manner. Assuming the cross-linked bonds to be hydrogen bonds, these workers analyzed the heats of formation data and estimated that a single cross-linking loci may consist of less than 10 or as many as 45 hydrogen bonds which contribute to differences in stability.

Concentration, molecular weight. Ferry and Eldridge (19, 23) have expressed rigidity of gelatin gels as a linear function of approximately the square of the concentration with slight deviations resulting from physical conditions and previous history. They also expressed the square root of the rigidity as a linear function of weight-average molecular weight. In an earlier study Ferry (21) reported rigidity is influenced more by preparation procedure than by the actual molecular weight. Lowe (39) said the setting time is shortened with increasing concentration; nevertheless 1.5 to 4 per cent is the normal range for food preparation.

Carrageenan

Carrageenan is a water soluble polysaccharide extracted from naturally abundant red seaplants. These hydrocolloids are strongly negatively-charged polymers of high molecular weight having unique and useful properties. It is now known that the carrageenan molecule consists

of two chemically different constituents, designated as kappa- (κ-) and lambda- (λ-) carrageenan, which are present in about equal amounts. The use of carrageenan in the preparation of blanc mange puddings, cough sirups, and hand lotions dates back to colonial times. The highly refined product now in widespread use is the result of advancements in manufacturing procedures spurred by the demand for substitutes for agar and gum arabic whose availability was sharply curtailed during World War II. The properties of any given carrageenan sample are a function of the method of manufacture, the previous thermal history, presence of electrolytes, pH, and the presence of other reactants.

Carrageenan source

Carrageenan is extracted from certain salt water plants, variously called Irish Moss or carrageen (rock moss), which are designated more exactly as Chrondus crispus or Gigartina stellata in the class Rhodophyceae or red algae. Idson (35) stated almost all the carrageenan processed in the United States is obtained from the Chrondus crispus. Red seaweed exist principally in both the Eastern and Western coastal waters; however they flourish in more temperate climates and are most abundant in the warmer waters off the Floridan, Californian, and Mexican shores. Abroad, red algae abound in the coastal waters of India, Malaya, Australia, and the Pacific Isles.

Manufacturing procedures

Harvesting. Chrondus crispus grows from a disc-like root which attaches itself to rocks in relatively shallow coastal waters. The moss is usually gathered by hand when the tide recedes or raked from below the

surface of the water (70). Seasonal variations make harvesting more profitable during certain times of year and give products with different gelation quality. The difference in products is apparently related to sexual maturity of the algae. Preliminary drying, resulting in 80 per cent moisture loss, is followed by controlled washings to leach out color pigments. According to Marshall and Orr (40) gelling quality of the product is improved by a storage period between these two steps.

Extraction. Carrageenan can be leached from the seaweed by water at various temperatures or it may be precipitated from a seaweed solution by alcohol.

The temperature of water for extraction has a pronounced effect upon the properties of the carrageenan sample. Fulton and Metcalf (26) reported the cold water fraction had 1/6 the gel strength of the hot water fraction. Data from three extraction temperatures, cold, 40 to 50°C; hot, 80 to 100°C; and pressure, 115 to 120°C, used by Rose (55) showed only the hot extract had gel strength. Furthermore, a 1 to 2 per cent solution of the hot extract gelled readily. More recently Goring and Young (30) found a certain quantity of gelation with extracts at 30, 60, and 100 to 120°C; however there was a pronounced maximum gel strength with the 60°C extraction. In conjunction with the fractionation of the two constituents of carrageenan, Smith and Cook (60) first extracted the solution at 60°C and then extracted the residue at 100°C. The fractions contained 64 and 26 per cent κ - and λ -carrageenan, respectively, at 60°C and 14 and 78 per cent at 100°C. Marshall and Orr (40) reported gel strength of the pressure extract could be improved

by treatment with an alkaline solution. An acid treatment hydrolyzes the extract rapidly destroying its gel forming capacity whereas a certain degree of alkaline hydrolysis produces a substance more sensitive to gelation.

Filtration, drying. Following extraction, the carrageenan solution is clarified and filtered. The product may be bleached with hydrogen peroxide; however this practice has been found to have a deteriorative effect upon the gelling power and viscosity of the carrageenan sol. The product is partially evaporated under vacuum and then either spray dried or completely vacuum dried. In the Lund process as reviewed by Tseng (69) addition of sugars such as sucrose or dextrose to the carrageenan solution has been advocated in order to protect carrageenan from deterioration during evaporation and drying. In this way the moisture content could be reduced to 2 to 3 per cent whereas without sugar protection, drying to 10 to 12 per cent gave a product susceptible to deterioration.

Composition

The ash-free polysaccharide is composed of D- and L-galactose, 3,6-anhydro-D-galactose, and sulfate ester groups. Rose (55) confirmed the earlier work of Haas and Russell-Wells (32) by showing hot and cold water extractions of Chrondus crispus gave two polysaccharides of different optical rotation although Buchanan et al (13) have proved the galactose portions of both to be similar.

The chemical heterogeneity of carrageenan has been supported by recent electrophoretic, sedimentation, and diffusion studies. Cook and associates (15) found the macromolecule was composed of two components,

one appeared to be linear and the other branched. These two components were separated by fractionation with potassium chloride by Smith and Cook (60). At approximately 0.15 M. potassium chloride one component precipitates as a gel, designated as the μ-carrageenan, which is removed from the unaffected fraction, λ-carrageenan, in the supernatant.

A complete methylation study of the structure of carrageenan is cited by Smith and Montgomery (62). This method positions the sulfate group on C_4 and supports the α 1-3 linkages with the possibility of some branching on C_6. These methylation studies were completed before the fractionation of the two components. Following this development Smith et al (61) reported the μ-portion contains D-galactose and 3,6-anhydro-D-galactose residues in ratio of approximately 1.4:1 together with 25 per cent esterified sulfate whereas the λ-portion contains D-galactose together with approximately 35 per cent esterified sulfate. These data are supported by the work of O'Neill (49, 50).

From infra-red and X-ray data, Bayley (8) proposed the stretched fibers of carrageenan all have fiber periods of 25.2 A. Furthermore, the fiber period of μ-carrageenan appeared to contain two trisaccharide units, each composed of two sulfated α-D-galactose residues linked α1-3 and one 3,6-anhydro-β-D-galactose residue linked 1-4 and, within a 25.2 A. period, a single side chain residue of 3,6-anhydro-D-galactose appeared to be linked through C_6 of a sulfated D-galactose unit in the main chain. The λ-carrageenan fiber period may represent 3 disaccharide units, the majority of which are composed of two 1-3αD-galactose sulfate residues. Smith and Montgomery (62) have diagramed the generally accepted chemical formula. Hansen and Whitney (33) disputed the

composition of the κ-carrageenan fiber period and have diagramed it slightly differently.

Reaction of carrageenan with milk

Carrageenan exhibits a specific reaction with milk which gives rise to an increased stabilization of suspended particles. Glabe and his associates (28) reported 0.03 to 0.04 per cent carrageenan is necessary to suspend one pound of cocoa in 100 pounds of milk whereas thirty times as much carrageenan is needed to suspend the same amount of cocoa in an equivalent weight of water. Rose and Cook (56) found the presence of carrageenan increases the viscosity of milk to a point where the particle size of ingredients such as cocoa is in a stable colloidal state. Whether the increase in viscosity is due to a casein-carrageenan complex or whether the carrageenan is associated with serum proteins is not fully known.

Smith (59) stated κ-carrageenan was more effective than λ-carrageenan in increasing milk viscosity. However, with potassium depleted milk, λ-carrageenan retained its original effect whereas κ-carrageenan's effect was greatly reduced. Moreover, the addition of potassium ions to milk did not greatly increase the effect of κ-carrageenan. The natural potassium content of milk (0.04N.) seemed to be sufficient to develop the full effect. Furthermore, the investigator found considerably more carrageenan was necessary to affect a comparable viscosity increase in 0.04 N. potassium chloride than in milk. From these data Smith concluded ionic bonding of the anionic hydrocolloid with the potassium ion did not account entirely for increased reactivity with milk.

Gel formation

Gel formation of carrageenan sols is a precipitation phenomenon, involving ionic bonding between certain metallic cations and the strongly negatively-charged anionic polysaccharide. Both monovalent and bivalent cations have the ability to form these ionic bonds although some are much more effective than others. Bivalent cations form gel structures by the formation of crosslinks between carrageenan chains whereas monovalent ions are involved in an intramolecular ionic bonding in which the ion is thought to actually fit into the crystal lattice of the carrageenan molecule. All carrageenan gels are thermally reversible. The exact temperature at which the sol-gel transformation takes place depends upon the concentration of ingredients present.

Gel strength

Strength or rigidity of carrageenan gels is affected primarily by temperature of sample extraction and by presence of electrolytes. In addition, gel strength is a function of the presence of other ingredients, pH, temperature, concentration, and molecular weight.

Presence of electrolytes. Although the presence of cations is essential to formation of gels, not all cations will produce gels of the same strength. Rice (53) noted potassium chloride was more effective than calcium chloride which, in turn, was more effective than sodium chloride in precipitating and setting carrageenan sols. Calcium salts produce a weak gel structure beyond which further salt addition has no effect (58). Gels formed in the presence of both potassium and calcium salts tend toward the strength produced by calcium ions with the gelling temperature

controlled by potassium ions. This conclusion has been supported by Marshall and Orr (40) who stated, in every case studied, potassium produced firmer gels than magnesium, sodium, ammonium, calcium, or lithium salts. Stoloff (64) observed the rank order of decreasing effectiveness of cations for gel strength development capacity is potassium, ammonium, calcium, magnesium, aluminum, and sodium.

In their studies on the fractional precipitation of the components of carrageenan by potassium chloride, Smith and Cook (60) found certain other monovalent cations, such as cesium or rubidium, were also effective gelling agents whereas sodium and lithium were not. Through further study, these investigators concluded the smaller ions fit into the crystal lattice of κ-carrageenan, the component involved in gelation, whereas the larger hydrated ions of sodium and lithium do not. None of the ions noted above affected λ-carrageenan.

Presence of other added ingredients. Tressler and Lemon (68) stated the presence of solutes in colloidal solutions of carrageenan compete with colloidal micelles for water resulting in altered colloidal properties. In general, the greater the quantity of solute present the greater the gel strength. The elastic texture of carrageenan gels prepared with other polysaccharides or mono- or disaccharides present indicates that weak hydrogen bonds are probably involved to some extent in these systems (58).

pH. Carrageenan gels are relatively unaffected by pH unless the system becomes acidic enough to cause hydrolysis and degradation of the polymer. This condition usually occurs at pH 3.5 or lower and is accentuated by heat (58).

Temperature. Carrageenan gels are thermally reversible and, in common with other thermally reversible gels, they melt at higher temperatures than those at which they were formed. Fulton and Metcalf (26) noted a 2 per cent gel melts between 54 and 60°C although it originally forms between 38 and 43°C. The gel strength is slowly reduced by high temperatures. Rice (53) stated gelation temperature is dependent upon the type and amount of salt present. Stoloff (64) reported if sufficient carrageenan was present to form a gel, the original gelling temperature increased with increasing concentrations of potassium chloride and was independent of the hydrocolloid concentration.

Concentration, molecular weight. Firm gels may be produced with as low as 1.0 per cent carrageenan solutions. Stoloff (64) stated, as the hydrocolloid is purified, the concentration must be increased or the temperature lowered to obtain a gel. Goring and Young (30) found, although there is a slight relationship, the gel strength of carrageenan gels was more dependent on structural factors than on the molecular weight of the sample.

Alginic Acid

Alginic acid is the ioniziable polysaccharide of high molecular weight extracted from naturally abundant brown algae. According to Steiner and McNeely (63), although the hydrophillic colloidal substance was discovered by Stanford in 1883, most of the commercial development and theoretical discoveries have materialized in the last few decades. Commercial production of algin began in California in 1929 and, due to the abundance and versatility of the product, has expanded until algin

is the most important natural water-soluble gum produced in the United States. The versatility of the product has led to its use as thickening, suspending, stabilizing, gel-producing, film-forming, and adhesive agents.

The term "algin" was first used by Stanford for the product which he later identified as sodium alginate. Since Stanford's time algin has been used as a common name for sodium alginate although some authors use the term to refer to all soluble salts of alginic acid or more vaguely to all salts of alginic acid and to alginic acid itself.

The gel formation of the alginates is dependent upon the presence of bivalent cations. In addition, the properties of algin samples are affected by their source, method of manufacture, previous thermal history, pH, and presence of electrolytes.

Alginic acid source

Alginic acid is extracted from a number of different species and genera all belonging to the class Phaeophyceae or brown algae in which alginic acid occurs, along with cellulose and other polysaccharides, in the cell wall. From data compiled for the First International Seaweed Symposium held at Edinburgh in 1952, the major sources of brown algae are reported as the American coastal waters of both Atlantic and Pacific Oceans and the coastal water of Northwest Europe including those of Norway, Great Britain, France, and Spain; while less prolific beds are located off Japan, Chile, South Africa, Australia, and New Zealand (35). These brown algae flourish in temperate and in polar zones; thus the giant kelp Macrocystis pyrifera, principal sources of alginates on the Pacific Coast, are found from Alaska to California whereas Laminaria

digitata, principal Atlantic sources, are located in coastal waters of Nova Scotia.

Manufacturing procedures

The grade or quality of the material is governed by temperature and duration of water and/or acid washes and the method of precipitation of calcium alginate. Fresh seaweed gives the best grade and predrying is deleterious unless carried out at room temperature. Black et al (10) observed that any form of thermal drying leads to depolymerization of high-grade alginate present in fresh seaweed. However, air-drying or drying in a vacuum dessicator over calcium chloride gave no loss of grade and drying over phosphorus pentoxide to almost anhydrous conditions lowered the grade slightly.

Harvesting. The giant kelp of the Pacific coast spreads out on the surface of deep water and can be harvested by ocean-going mechanical barges. The Laminaria grows under the water's surface and is gathered by motor boats equipped with grappling hooks operating at depths of 10 to 20 feet. A small quantity is gathered as driftweed after a storm or raked from the bottom in shallow waters (73).

Although a number of manufacturing processes have been developed since the Stanford process was first used in nineteenth century Scotland, the two processes most commonly used in the United States are the Algin Corp. process and the Kelco process.

Algin Corporation process (68). In this process, patented by Le Gloahec and Herte in 1938, either fresh or dried Laminaria is soaked in a

solution of calcium chloride. At the end of the soaking period the seaweed is first washed with water, then with dilute hydrochloric acid to remove any residual salts, and finally with water. The seaweed is digested with warm dilute sodium carbonate for approximately 3 hours at room temperature with continuous agitation. The mass is diluted to form an emulsion which, after the cellulose has been removed, yields a crude solution of sodium alginate. The crude product is decolorized, clarified, and precipitated with hydrochloric acid to give alginic acid. This alginic acid may be dried at 140 to 160°F (60 to 71°C) or it may be converted into alginates before shipping.

Kelco process (68, 69). This process, originally patented by Green in 1936, avoids heating the solutions over 50°F (10°C). Freshly harvested Macrocystis pyrifera is leached for several hours with weak hydrochloric acid to reduce the salt content. The acid liquor is drained, then the chopped or shredded kelp is digested at pH 10, brought about by sodium carbonate addition, to give a gelatinous mass. Following a second soda ash digestion, the product is completely disintegrated in a hammer mill. This solution is clarified and filtered. The filtrate, containing sodium alginate, is then treated with 10 per cent calcium chloride to precipitate the alginic acid as a calcium salt. The curdlike calcium alginate is separated, washed, and bleached. It is then converted into alginic acid by addition of hydrochloric acid. Repeated washings constitute the final step; however alginic acid prepared in this way is somewhat unstable and must be stored under refrigeration. Consequently it is frequently converted into a salt which can be dried for shipment.

Composition

Until recently alginic acid was generally accepted as a linear polymer of anhydro-β-D-mannuronic acid of high molecular weight linked in such a way that each anhydromannuronic acid unit had one free carboxylic acid group and two free hydroxyl groups. This concept of structure was derived from fundamental investigations of Nelson and Cretcher (17, 45) in the late 1920's. Chanda et al (14) reported results from studies carried out on less degraded alginic acid which confirmed the view that the main structural feature of the alginic acid molecule to be a chain of 1,4-linked-β-D-mannuronic acid residues. X-ray analysis by Astbury (7) had shown that the stretched fibers gave a well defined diffraction pattern, indicating a high degree of orientation. Interpretations of these data agreed with the concept of alginic acid as a linear chain. More recent chromatographic studies done by Fischer and Dorfel (24) showed alginic acid was composed of L-guluronic as well as D-mannuronic acid. Therefore more research will have to be done before the structure of the alginic acid chain can be stated.

Gel formation

Gelation of alginate sols is a precipitation reaction with cross-bonding occurring between a negatively-charged alginate anion and a positively-charged cation supplied by the gelling agent. The gelation process for alginate sols is not thermally reversible. Idson (35) reported sodium alginate solutions will form gels with acids or with calcium salts.

The metallic cation gel structure has been studied by a number of investigators. Mongar and Wassermann (43) assume the main valency chains

in the stretched calcium alginate fibers are attached to each other in a broadside-on position by salt bridges operating between the bivalent calcium and the carboxyl groups of the polyanion. This formation of crosslinks implies that segments of adjacent alginate chains are relatively straight and approximately parallel to each other. A recent investigation of Thiele and Andersen (65) supported the bonding theory proposed. Moreover, they stated the orientation of the gel structure, due to reproducible directed coagulation in converting the sol into a gel, is influenced by the direction of flow of diffusing ions and by the ion itself. A high degree of orientation is associated with dehydration of the colloidal particles and with a small capacity for swelling. Thiele and Hallich (66) in a later study of the ionotropic gel formation of sodium alginate sols by the diffusion of ions found, under suitable conditions, drops of water segregated. As gel formation progressed in the direction of diffusion these liquid drops became tapering capillaries that were straight, parallel, and often nearly identical with one another. The exact structure and size of the capillaries were specific to the diffusing metal ion and were related to the degree of ionotropic orientation produced by the ion.

Gel strength

The gel strength or rigidity of an alginate gel depends largely upon the presence of electrolytes. These gels are also influenced to some extent by pH and concentration. Unlike the other gel systems previously reviewed they are not thermally reversible and do not require chilling for gelation to occur (41).

Presence of electrolytes. Gelation of alginate sols requires the presence of certain metal cations. Thiele and Andersen (65) reported differences in the degree of orientation of ionotropic gels due to the directing ability of the gelling cation. This directing ability decreases from lead to copper to cadium to barium to calcium. Scott (57) presented another phase in the effect of electrolytes upon gel strength of polycarboxylates such as the alginates. Complexes which had been formed by polyanions with cationic detergents were soluble in salt solutions at concentrations characteristic to the structure of the polyanion polymer. For alginates 0.33 N. potassium chloride or 0.30 N. magnesium chloride had sufficient ionic strength to maintain 70 per cent of the polysaccharide in solution.

pH. At extremes of the pH scale alginate solutions will thicken and form gels without the addition of bivalent cations. Algin solutions are unaffected by pH ranging from 4.5 to 12. Below pH 4.5 the solutions thicken, gel, and finally at pH 3 alginic acid is precipitated. The processor (2) stated the alginates with the exception of propylene glycol alginate which is resistant to both excessive acid or alkali, will thicken and form weak gels at pH's above 12.

Concentration. Steiner and McNeely (63) proposed firmness of alginate gels is a function of both the concentration of the alginate and the bivalent ions present. Gibsen and Rothe (27) stated 0.8 per cent sodium alginate gives desirable dessert gels or milk puddings.

Commercial Applications of Gelling Agents

The most important quantitative use of gelatin in the food industry is in the manufacture of gelatin desserts. Gelatin is also used extensively in the stabilization of marshmallow foams. In baked products gelatin acts as a stabilizing agent in chiffon-type fillings thus preventing syneresis; and in icings, particularly the boiled type, it not only serves as a setting material but has some influence on sugar crystallization. In the dairy industry gelatin stabilizes ice cream by preventing crystal growth. Gelatin is also used in the preparation of jellied meats and madrilene soups. Idson and Braswell (36) ranked capsule preparation first in the use of gelatin in the pharmaceutical industry. In pills, tablets, and lozenges gelatin has found application as a binding agent and for coating and glazing purposes. Stable emulsions of mineral oils, caster oil, and vitamin-containing fish oils are among the many prepared with gelatin as the emulsifying agent.

Feeding tests (47) have shown carrageenan to be completely harmless in amounts normally used in foods and it has been accepted as a safe food additive by the Federal Food and Drug Administration (20). The first major use of carrageenan in foods was for the stabilization of cocoa particles in chocolate milk. A more recent list of uses presented by Glabe et al (29) includes the strengthening of wheat gluten to produce a firmer spaghetti, the texture improvement of white cakes, and the production of quick-drying icings. Various carrageenan types have been recommended (58) for use in stabilizing whipping cream and ice cream, producing milk-base puddings and pie fillings, producing water-type dessert gels, stabilizing and thickening fruit pie fillings, stabilizing hand lotions and cough sirups, and emulsifying mineral oils.

Algin has been eaten for hundreds of years as a constituent of kelp while algin itself has been used in various foodstuffs for a quarter of a century. Extensive animal feeding tests (46, 51) have shown that algin is nontoxic and not an allergen. According to Gibsen and Rothe (27) propylene glycol alginate has found extensive use as a thickening and emulsifying agent in French dressings and is one of the optional emulsifying agents listed by the F.D.A. Steiner and McNeely (63) enumerated a number of other uses. The addition of algin to milk in special formulation produced a soft custard-type pudding. Fruit juice gels have been prepared by the addition of calcium ions and algin. Formula adjustments produce firmer gels useful in candies. In Germany some interest has been shown in the use of algin films as edible coatings, such as sausage coatings. Algin can also be used to produce nonsticky icings and glazes. Steiner and McNeely also stated that algin is the most important ice cream stabilizer in the United States and Great Britain. Differences among the three gelling agents in stabilization of ice cream has been discussed by Potter and Williams (52) who reported algin produced no viscosity change in the ice cream mix and eliminated the necessity for aging whereas gelatin increased viscosity and required aging and carrageenan produced such a high initial viscosity that cooling was difficult. In the pharmaceutical industry algin has found extensive use as a tablet-disintegrating agent. Numerous investigators reported algin's use as thickening and stabilizing agents in ointment bases, suspensions, and emulsions.

Gel Strength Measurements

Various tests have been developed to measure the strength of gels. Most of them depend upon measurement of force necessary to erupt the gel structure for a specified distance or the distance erupted by a specified force. In contrast the Exchange Jelometer measures the natural elasticity of the gel. The measurement of strength of gelling agents is standard procedure for their manufacturers. In fact, the Bloom gelometer test is so universally applied to testing of gelatin that grades of gelatin are designated as various Bloom values.

Bloom gelometer (12, 67)

The Bloom gelometer test measures the weight necessary to force a 12.7 mm. round plunger 4 mm. into a gel which has been prepared under standardized conditions. Depending upon the grade of gelatin either a 6.66 or a 12.5 per cent solution is prepared and held at 10°C for 17 hours before testing. It is generally accepted that results of a 6.66 per cent concentration are expressed as blooms and those of a 12.5 per cent concentration as double blooms. Values obtained from gelatin samples range from 0 to slightly over 300 grams and each gram constitutes a bloom.

Boucher jelly tester (38)

The Boucher test measures the force in milliliters of water required to make a 5 mm. depression by a plunger 13 mm. in diameter into a standard gel. The 5 per cent gelatin solution is held at 10 ± 1°C for 16 to 18 hours. This test is performed within two minutes after the gelatin gel is removed from the constant temperature bath. A force of

one milliliter of water is designated a 1 jellogram. The full normal range extends from 4 to 500 jellograms. To equate the Boucher value to Bloom values the following formulas are used:

$$\text{Bloom } (6.66\%) = \text{Boucher} \times 2/3 + 18$$

$$\text{Bloom } (12.5\%) = \text{Boucher} \times 7/3 + 46$$

Tarr-Baker jelly tester (37)

The Tarr-Baker jelly tester applies pressure to a plunger of known area, which is resting on the surface of a standard gel. The pressure is read at the instant the gel breaks. In preparation of the gel its surface is covered with a light film of mineral oil to prevent skin formation. Following aging, the oil is removed and the tester's plunger is situated over the gel. Pressure applied by a flow of water is controlled so the manometer column rises 60 cm. per minute. The manometer is read when the gel breaks. Tests are made in triplicate and averaged and gel strength is reported as the height (in centimeters) reached by the liquid in the manometer at the gel's breaking point, for a given concentration.

Fuchs penetrometer (37)

This test measures the time required for a plunger of a given weight to cut into the gel to a specified depth. A standard gel is prepared and following aging the top 1/4-inch layer of gel is removed. The plunger of the Fuchs penetrometer is adjusted so that it rests on the top surface of prepared gel. A weight is imposed on the plunger which is held in a true vertical position. The plunger itself is a sharpened hollow metal tube that is highly polished. The plunger is released and

time, in seconds, required for the plunger to cut into the gel to a certain depth is noted. This depth is determined by markings on the rod or shaft affixed to the plunger. Time required to reach two different depths is noted for each test.

Exchange jelly tester (16)

The Exchange jelly tester measures the amount of natural elasticity which a gel possesses. This is accomplished by pouring a gel to a standard depth of 3.125 inches and carefully inverting the gel onto the instrument which is fitted with a microscrew whose one complete revolution lowers the screw 0.03125 inches or 1 per cent of the original height. Thus by adjusting the screw so the tip just touches the released jelly per cent sag can be determined directly. This test is unique in that it does not rupture the jelly and does not strain it beyond its normal elastic limit. Reproducibility of the test is considered good.

Recording gel tester (34)

This test is a mechanized modification of the Saare method. In this test a device lowers at a slow uniform rate a gel containing an embedded disc which is attached by a cord to a dynamometer capable of measuring the desired rate of force. As the gel is slowly lowered, the dynamometer reacts upward with a continuously increasing force on the disc until the yield point of the gel is reached. (The dynamometer-recorder mechanism of the Corn Industries Viscometer can be adapted to record this force.) The load can be read in gm-cm. from the dynamometer or can be converted into grams. The reproducibility of the test is good; the average variation in tests of replicable gels did not exceed ± 5 per cent of the mean yield point.

PROCEDURE

Three types of gelling agents were arbitrarily selected for this study: acid-processed, pure, unflavored gelatin with a bloom value of 220; carrageenan;[1] and algin preparation.[2] These products are recommended by their manufacturers for use as efficient gelling agents in milk-type desserts.

Preliminary Investigation

The amounts of carrageenan and of algin preparation required to produce gel strength equivalent to that of pure gelatin in a whole milk-cornstarch system were determined through preliminary study.

The milk-cornstarch system consisted of 946 milliliters (32 ounces) of reconstituted milk, prepared from 127.6 grams of whole milk solids and 887 milliliters (30 ounces) of 40 to 50°C tap water, and 13.5 grams of cornstarch. Previous investigation indicated that the addition of 14 grams of pure gelatin produced desirable gel consistency for this system. Varying amounts of carrageenan and of algin preparation were tried until each of these agents produced a gel comparable to the product obtained with 14 grams of gelatin. A Precision Penetrometer (Arthur H. Thomas Co.) with 150-gram load cone attachment was used to establish equivalent gel strength among products.

For test samples prepared with gelatin or with carrageenan 829 milliliters (28 ounces) of milk was placed in a double boiler and heated to 80°C. In the preparation of the gelatin samples the gelatin was hydrated in 58.5 milliliters (2 ounces) of cold milk and dissolved over hot water. The cornstarch was dispersed manually in the remaining

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[1]Type 10 Carrageenan manufactured by Marine Colloids, Inc., Chicago 5, Illinois.

[2]Margel, a mixture of algin and calcium carbonate, manufactured by Kelco Company, Chicago 6, Illinois.

58.5 milliliters (2 ounces) of cold milk. The two mixtures were combined, blended, and added to the heated milk. For the carrageenan samples the cornstarch and carrageenan were dry-blended manually, dispersed by the gradual addition of 117 milliliters (4 ounces) of cold milk, and then added to the 80°C milk.

The procedure used for the algin preparation samples differed slightly. Preliminary trials indicated the algin preparation could be dispersed more efficiently in hot milk than in cold. Therefore, all of the milk was heated to 75°C. The algin preparation and cornstarch were dry-blended manually, sprinkled on the surface of the hot milk, and incorporated with a French whip.

All mixtures resulting from the addition of cornstarch and gelling agent were held at 77±1°C for fifteen minutes with controlled intermittent stirring. At the end of each cooking period samples were poured into coded 5-ounce pyrex custard cups to a designated depth, covered with Saran wrap, and refrigerated at 3 to 6°C for 20 to 24 hours before penetrometer measurements were taken.

From this investigation it was found that 2.5 grams of carrageenan or 12 grams of algin preparation produced gel strength equivalent to 14 grams of pure gelatin in the milk-cornstarch system studied. Penetrometer values for the six replications for the three gelling agents and the analysis of variance for these values appear in the Appendix.

If the weight of the gelatin required is considered unity, 0.179 times as much carrageenan or 0.857 times as much algin preparation may be substituted to produce equivalent gel strength in the milk-cornstarch system described.

Basic Formula

The basic formula was developed through preliminary study of products made from selected milk-type dessert formulas in which pure gelatin was used for the gelling agent. Only formulas in which whole egg and cornstarch constituted a part of the thickening effect were considered.

The basic formula consisted of constant proportions of whole milk solids, whole egg, cornstarch, salt, and water:

Ingredients	Amount
Whole milk solids	510.4 grams
Whole eggs	486.0 grams
Cornstarch	54.0 grams
Salt	6.0 grams
Water	3784.0 milliliters (128.0 ounces)

Formula modification

This basic formula was modified by the addition of 56 grams of pure gelatin for Series 1, 10 grams of carrageenan for Series 2, and 48 grams of algin preparation for Series 3. The amounts for carrageenan and for algin preparation were based on the proportional weight of gelatin required by these agents to produce comparable gel strength as determined by preliminary study.

Each series was further modified by the type and concentration of sweetener added: (a) 275 grams and 735 grams of dry sucrose, based on 20 and 40 per cent of the total weight of the modified formula exclusive of water, and (b) 9.17 grams and 24.5 grams of dry calcium cyclamate, based on the standard sucrose-calcium cyclamate sweetness equivalent for

the sucrose concentrations selected for the study (31). A control for each gelling agent was also prepared without sweetener.

In the experimental design presented in Table 1, the concentrations of sweeteners for Series 1, 2, and 3 are designated as A, B, C, D, and E for the control, 20 per cent sucrose, 40 per cent sucrose, calcium cyclamate equivalent to 20 per cent sucrose, and calcium cyclamate equivalent to 40 per cent sucrose, respectively. Proportions of ingredients for the fifteen experimental treatments are presented in the Appendix.

Table 1. Design of experiment.

Sweetening Agent Type and Concentration	Gelling Agents		
	Series 1 Gelatin	Series 2 Carrageenan	Series 3 Algin Preparation
A. Control	X	X	X
B. Sucrose -- 20%	X	X	X
C. Sucrose -- 40%	X	X	X
D. Ca. cycla. = 20% Sucrose	X	X	X
E. Ca. cycla. = 40% Sucrose	X	X	X

Ingredient Procurement

Where possible, the materials used in this study were obtained from common lots and, unless otherwise noted, were kept in dry storage at room temperature.

Basic formula ingredients

Whole milk was spray-dried by the Michigan State University Dairy, stored in closed polyethylene bags at -20°C until approximately a week before its use, at which time it was transferred to refrigerator storage at 3 to 6°C.

Fresh eggs were obtained from the same flock of White Leghorn hens at the Michigan State University Poultry Farm. The eggs were mechanically blended, packed in covered plastic-coated containers, quick-frozen at -40°C for 48 hours, and stored at -20°C until needed.

The cornstarch and salt were obtained from the Michigan State University Food Stores and were stored in their original fiber containers.

Cold water, in which the natural hardness had not been altered by chemical means, was used in this study. A standard chemical test[1] was used to determine the degree of hardness of the water used in each replication.

Gelling agents

The gelatin was obtained from the Michigan State University Food Stores and stored in its original fiber container with metal top. Carrageenan and algin preparation were obtained from Marine Colloids, Inc. and Kelco Company, respectively. Both products were stored in closed polyethylene bags in their original cardboard drums.

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[1]A Model 69 Kit, Hach Chemical Company, Ames, Iowa, was used to test for water hardness. The author gratefully acknowledges the assistance of Angela Hoxey, Research Technician, Michigan State University Dairy Plant, in the determination of water hardness for this study.

Sweetening agents

Superfine sucrose was obtained from the Michigan State University Food Stores and stored in closed polyethylene bags in airtight metal containers. The entire lot of calcium Sucaryl[1] was supplied by the Abbott Laboratories and was stored in its original brown-glass container.

Batch Size

All modifications of the basic formula yielded approximately one gallon of custard. Preliminary work with both quart and gallon batches showed no significant difference in gel strength as measured by a precision penetrometer for a given series at a particular type and concentration of sweetener. In order to present findings which might be helpful to institutional users as well as homemakers, gallon batches were prepared throughout the study. Four replications of each treatment were prepared.

Prepreparation of Ingredients

Whole dried milk, sufficient for one day's preparation, was weighed on a torsion balance with a 4.5-kilogram capacity, reconstituted with 40 to 50°C water, stored in a 5-gallon milk can, and refrigerated at 3 to 6°C for 48 hours prior to use. The frozen eggs were defrosted for 48 hours in a refrigerator at 3 to 6°C and held at room temperature 4 hours prior to use.

Each day's preparation consisted of three batches of custard representing one replication of each gelling agent at one level of sweetener. All ingredients were weighed or measured on the day of preparation. The sucrose, whole egg, cornstarch, and salt were weighed

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[1]Abbott Laboratories' registered name for cyclamate.

on a torsion balance with a 4.5-kilogram capacity whereas the gelling agents and calcium cyclamate were weighed on a torsion balance with a 2.0-kilogram capacity. To facilitate removal and to minimize handling losses the gelling agents and the calcium cyclamate were weighed on glazed black weighing paper and transferred with the aid of a narrow rubber spatula. The fluid milk as well as the water used for reconstitution were measured in 500- and 2000-cubic centimeter graduated enamel pitchers.

Cooking Process

Immediately preceding the cooking of each replication, the relative humidity was determined from the difference in dry- and wet-bulb temperatures read on a Tyeos Humidiguide.

A ten-quart Groen Steam Kettle, Model TDB, was used in the preparation of all products tested. Steps in the cooking procedures for Series 1, 2, and 3 were kept as similar as feasible and are presented in Table 2. Differences among dispersion methods for the selected gelling agents, as established through preliminary trials, necessitated slight procedural variations. End cooking temperatures of 74°C for the control samples and for those made with both concentrations of calcium cyclamate, and 80°C for the 40 per cent sucrose samples were established through preliminary study of the effect of sucrose and calcium cyclamate on the coagulation temperature of the egg protein in the basic formula. The end cooking temperature for the 20 per cent sucrose samples was arbitrarily set at 77°C, half way between the temperature established for the control and for the 40 per cent sucrose samples.

Step	Series 1 Pure Gelatin	Series 2 Carrageenan	Series 3 Algin Preparation
1	Reconstituted whole milk (3134 ml.) heated to 80°C in 10-qt. Groen Steam Kettle.	Reconstituted whole milk (3134 ml.) heated to 80°C in 10-qt. Groen Steam Kettle.	Reconstituted whole milk (3458 ml.) heated to 75°C in 10-qt. Groen Steam Kettle.
2	Gelatin hydrated with 162 ml. cold milk and dissolved over hot water. Cornstarch, salt, and sucrose or dry calcium cyclamate when appropriate, dry-blended,[a] dispersed in 162 ml. of cold milk, and combined with dissolved gelatin.	Powdered carrageenan dry-blended with salt, cornstarch, and sucrose or dry calcium cyclamate when appropriate, and dispersed by gradual addition of 324 ml. of cold milk.	Powdered algin preparation dry-blended with salt, cornstarch, and sucrose or dry calcium cyclamate when appropriate.
3	Dispersion formed in Step 2 added to 80°C milk and mixture held at 77±1°C for 15 min. with controlled intermittent stirring.	Dispersion formed in Step 2 added to 80°C milk and mixture held at 77±1°C for 15 min. with controlled intermittent stirring.	Dry-blended mixture sprinkled on top of 75°C milk and blended. Resulting mixture held at 77±1°C for 15 min. with controlled intermittent stirring.
4	Defrosted eggs blended thoroughly with 324 ml. of cold milk and added at end of Step 3. Final mixture reheated to designated end point[b] with occasional stirring.	Defrosted eggs blended thoroughly with 324 ml. of cold milk and added at end of Step 3. Final mixture reheated to designated end point[b] with occasional stirring.	Defrosted eggs blended thoroughly with 324 ml. of cold milk and added at end of Step 3. Final mixture reheated to designated end point[b] with occasional stirring.

[a]All blending and stirring done manually using a French whip.

[b]End cooking point: 80°C for 40% sucrose, 77°C for 20% sucrose, and 74°C for control and for both levels of calcium cyclamate.

Throughout the heating and cooking periods time-temperature relationships were recorded on a Minneapolis-Honeywell Type 153 Electronik Multipoint Recorder. Three thermocouple leads, taped together to form an equilateral triangle measuring approximately 1-inch per side, was centrally positioned in the cooking mass in the Groen Steam Kettle. The purpose of the triple readings was twofold: (a) to give three readings during each 96-second interval throughout the heating periods (i.e., throughout the heating of the milk and the heating of the entire mixture after the addition of the eggs) in order to assure exact end points, and (b) to serve as a check on the accuracy of the thermocouple leads themselves during the constant-temperature cooking period.

Throughout the preparation all dry-blending, dispersing, and stirring were done manually with a French whip. During the 15-minute cooking period stirring was controlled so that the mixture was stirred for two minutes and then allowed to rest for two minutes. This intermittent stirring ended with a 1-minute rest period. All timing was done with a stop watch.

Preparation of Samples

As soon as the custard reached the designated end temperature, the cooked mixture was poured through a medium-fine, wire-mesh household strainer directly into a calibrated container for measurement of batch depth. In calibrating this container the investigator determined the container depth in millimeters for volumes ranging from 150 to 190 ounces at 2-ounce intervals. A table was constructed from these data for the conversion of mass depth to mass volume.

As quickly as possible, 12 portions of the cooked custard were poured into coded, 5-ounce pyrex custard cups to a measured depth of 1 3/4 inches and individually covered with Saran wrap secured with a rubber band. This handling procedure required approximately 15 minutes. All samples were refrigerated at 3 to 6°C for 19 to 20 hours before objective evaluation.

Objective Measurements

On the day following preparation, objective tests were made to determine the gel strength, measured both as the ability to resist penetration and as the ability to hold a rigid shape; syneresis; and pH. All samples were taken directly from the refrigerator and the Saran wrap removed. The custard was loosened carefully with a thin, flexible, 1-inch stainless steel spatula.

Penetration

Gel strength, as measured by resistance to penetration by a falling force, was determined by a Precision Universal Penetrometer with a 5-second penetration for a 150-gram load cone attachment. This polished, balanced, brass cone had an elongated sharpened tip. The 150-gram load, which meets the A. S. T. M. standards, consisted of 102.5 grams and 47.5 grams for the cone and the shaft, respectively.

The prepared sample was placed on the leveled platform of the penetrometer so that the tip of the cone was centered over the custard. With the penetrometer dial set at zero, the cone and shaft assembly was lowered manually until the tip of the cone just touched the surface of the custard. This assembly was then allowed to fall free for a period

of five seconds and the amount of penetration recorded to the nearest 0.1 millimeter. Figure 1 shows the measurement of penetration after the release of the cone as well as the equipment used in this test.

Three samples from each replication were tested and the measurements averaged for the penetrometer value for that replication.

Per cent sag

In order to compare the firmness of custards, measurements of the height of the inverted sample before and after a specified time interval were needed. The apparatus shown in Figure 2 was developed to minimize damage to the gel structure of the sample during testing and to facilitate the collection of such data.

The glass surface, on which the sample was to be inverted, was positioned level with the zero-point of the ruler mounted at the left of the apparatus. The knurled vertical bar, mounted at the right of the apparatus, permitted adjustment of the height of the horizontal bar. Both the base of the apparatus and the horizontal bar were leveled periodically throughout the study.

The loosened custard was inverted on the glass surface of the apparatus and the horizontal bar lowered until it just touched the top of the custard as illustrated in Figure 2. After the initial height of the sample was recorded to the nearest 1/32 inch, the horizontal bar was raised so that it no longer touched the custard and the sample was allowed to stand undisturbed at room temperature for 15 minutes. At the end of this period the horizontal bar was lowered until it touched the sample and the height was recorded.

Figure 1. Measuring penetration after release of 150-gram cone attachment.

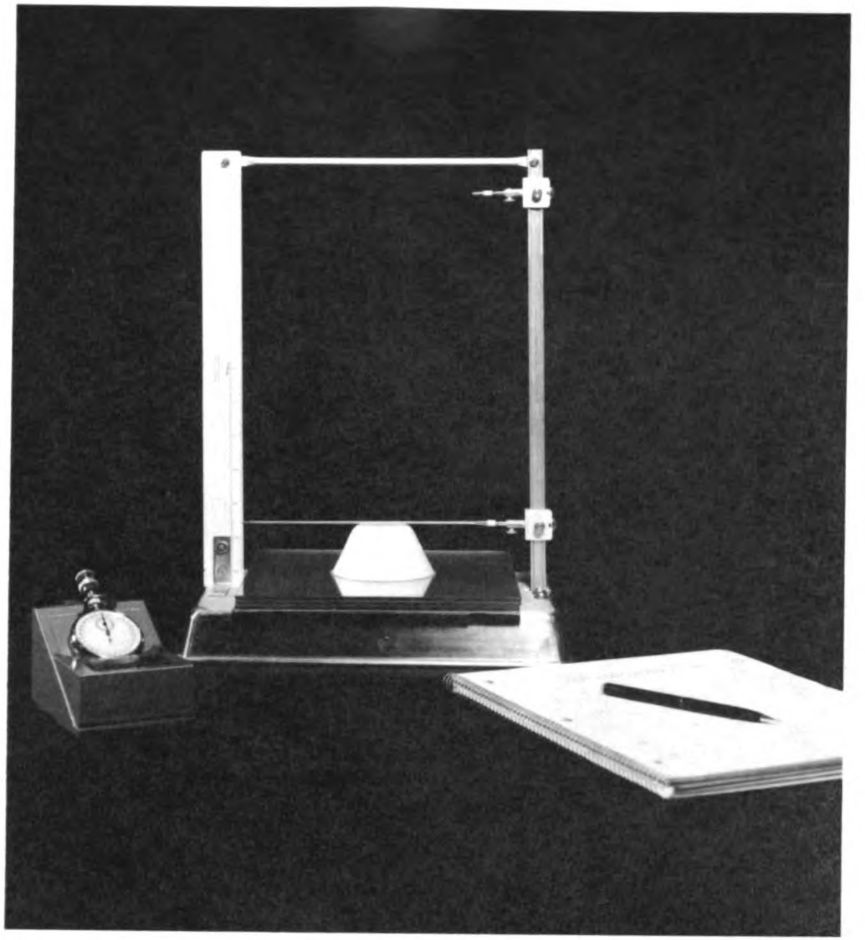

Figure 2. Apparatus for height measurement of free-standing inverted jellied custard.

Three samples from each replication were tested in this manner. The per cent sag for each sample was calculated from the difference between readings divided by the initial inverted height. These percentages were averaged to determine the per cent sag for the replication.

Syneresis

The method of Miller et al (42) was used to determine the weight of drainage of the custards. The equipment required for this test is shown in Figure 3.

The jellied custard was inverted on fine wire screening (15 wires per inch) which had been placed over a petri dish of known weight. The assembly was immediately covered with a stainless steel bowl to prevent evaporation and allowed to stand 30 minutes. At the end of this period the wire screening, containing the custard, was removed. The weight of the drainage, recorded to the nearest 0.01 gram, was equal to the difference between the weight of the petri dish before and after testing. Drainage weights of three samples from each replication were averaged to determine the value for the replication.

pH

Hydrogen ion concentrations were determined for triplicate samples of raw egg slurries on the day of preparation and for triplicate samples of jellied custard slurries on the day of objective evaluation as well as for duplicate samples of the distilled water used in the preparation of them.

Figure 3. Equipment used for drainage weight determinations from free-standing inverted jellied custards.

The egg slurry was prepared by blending manually 15 milliliters of defrosted whole egg with 60 milliliters of distilled water. Twenty-five grams of the jellied custard was blended manually with enough distilled water to bring the total volume to 75 milliliters. All samples for the pH determinations were used at room temperature (22 to 28°C) and were tested on a Beckman Zeromatic pH Meter with a glass and a saturated calomel electrode.

Analysis of the Data

The data were analyzed according to procedures recommended by the statistician of the Michigan Agricultural Experiment Station. Analyses of variance were used to evaluate differences attributable to treatments for penetration, per cent sag, and syneresis. Significant differences among treatments were evaluated through use of the Studentized range table.[1]

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[1]Duncan, D. Multiple F test. Journal of Biometric Society 2: 3-4, March, 1955.

RESULTS AND DISCUSSION

This study was directed primarily toward the objective measurement of gel strength and syneresis of jellied custards prepared with three gelling agents and two concentrations of two types of sweeteners. The treatment variables were fifteen in number and consisted of all possible combinations of gelling agents and sweetener concentrations as well as a control for each gelling agent prepared without sweetener.

Control of techniques used in the preparation of the samples was as complete as possible. In addition, data relative to water hardness, relative humidity of the laboratory during preparation, time and temperature of refrigerated storage, pH of raw egg and jellied custard samples, and batch yield were collected. Time-temperature relationships during cooking were recorded. Variations in these data were noted and considered in the interpretation of the findings of the study.

Water Hardness and Physical Factors

Among treatments the mean hardness of water used for reconstituting the milk solids varied from 299.3 to 312.1 parts per million. Mean values per treatment for relative laboratory humidity and time and temperature of refrigerated storage, based on four replications, ranged from 50.0 to 64.2 for relative humidity and 19.3 to 19.6 hours of storage at 3.3 to 4.4°C. These data show only minimal variation among treatments for each factor and are presented in the Appendix.

pH

Irrespective of the type and concentration of sweetener used, the pH ranges of jellied custards prepared with pure gelatin, carrageenan, and algin preparation were 6.4 to 6.9, 6.6 to 7.1, and 6.9 to 7.3, respectively. The pH ranges for individual treatments are reported in the Appendix. All samples were considered neutral although, within each gelling agent series, custard samples sweetened with calcium cyclamate exhibited slightly lower pH values than the control samples and those sweetened with sucrose.

Idson and Braswell (36) reported 9.1 as the isoelectric point of acid-processed gelatin. Boedtker and Doty (11) stated that gelatin gels formed at the isoelectric point in the absence of salts are weak; however if sufficient salts are present or if the pH is more than one unit away from the isoelectric point, weak gels are no longer formed. Since the gelatin custard formulas for this study contained a quantity of electrolytes and the pH of all samples was below 7.0, it seemed likely that pH did not affect the gel strength of these samples. According to their processors (2, 58), carrageenan gels are relatively unaffected by pH unless the solutions are extremely acidic and alginic acid gels are unaffected within a pH range of 4.5 to 12. In view of the findings reported by these workers, it was concluded that pH of the mixture did not affect the gelling strength of the selected gelling agents.

Batch Yield

Mean batch yield for treatment, sweetener conglomerate averages, and per cent volume increase, based on control batch yield, are reported in

Table 3. Average per cent volume increases for samples prepared with 20 per cent sucrose, 40 per cent sucrose, calcium cyclamate = 20 per cent sucrose, and calcium cyclamate = 40 per cent sucrose were 5.55, 11.51, 2.85, and 2.10, respectively.

Table 3. Treatment means and sweetener conglomerate averages for batch yield of jellied custard.

Sweetening Agent Type and Concentration	Gelling Agents: Gelatin Vol.	Gelatin Vol. Inc.	Carrageenan Vol.	Carrageenan Vol. Inc.	Algin Preparation Vol.	Algin Preparation Vol. Inc.	Sweetener Average Vol.	Sweetener Average Vol. Inc.
	Qt.	%	Qt.	%	Qt.	%	Qt.	%
Control	4.91[a]		4.92		4.94		4.92	
20% Sucrose	5.23	6.52	5.20	5.69	5.16	4.45	5.20	5.55
40% Sucrose	5.52	12.42	5.48	11.38	5.47	10.73	5.49	11.51
Ca.cycla.=20%Suc.	5.08	3.46	5.06	2.85	5.05	2.23	5.06	2.85
Ca.cycla.=40%Suc.	4.97	1.22	5.03	2.24	5.08	2.83	5.03	2.10

[a]Based upon four replications.

Analysis of variance of batch yield, presented in Table 4, showed significant difference attributable to sweetener concentration, at the 1 per cent level of probability. In a comparison of conglomerate averages of batch size for concentrations of sweetener, differences were established as follows: 40 per cent sucrose-sweetened custard volume > volumes of custards made with all other sweeteners. Twenty per cent sucrose-sweetened custard volume was greater than custard volumes of non-sweetened and the higher concentration of calcium cyclamate-sweetened samples. There was no significant difference, 1 per cent level of probability, between batch yields of custards sweetened with both concentrations of calcium cyclamate and non-sweetened custards and between batch yields of 20 per cent sucrose-sweetened custards and custards sweetened with the

lower concentration of calcium cyclamate. Inasmuch as differences in volume affect the dilution of the gelling agent, it is conceivable that differences in gel strength may be due, in part, to batch yield.

Table 4. Analysis of variance for batch yield of jellied custards.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Squares
Total	14	0.5850	
Gelling Agents	2	0.0000	0.0000
Sweeteners	4	0.5741	0.1435**
Error	8	0.0109	0.0014

**Significant at 1 per cent level of probability.

With the exception of two treatments, gelatin or carrageenan in combination with calcium cyclamate = 40% sucrose, the orderly relationship between weight of sweetener and batch yield appeared to account for volume increase. However, when the mean batch yields of samples made with combinations of gelatin or carrageenan with both concentrations of calcium cyclamate were compared, the relationship between weight of sweetener and batch yield was reversed. Although this reversal of volumes is less pronounced for carrageenan samples than for gelatin samples, this finding suggests that the higher concentration of calcium cyclamate may have affected the gelling capacity of these agents.

Time-Temperature Relationships During Cooking

The cooking curves for each treatment were divided into three periods: the heating curve of the milk, the 15-minute holding period

at 77±1°C after the addition of gelling agent and sweetener, and the heating period after addition of the egg-milk mixture.

Heating curves of milk

The heating curves of milk for all treatments showed only minimal variation. The average heating times for both the gelatin and carrageenan series, heated to 80°C, was 9.0 minutes and for the algin preparation series, heated to 75°C, average heating time was 8.3 minutes. The rate of heating was controlled so approximately 5 pounds of pressure were maintained on the 10-quart Groen Steam Kettle. The thermostat was set at 200°F until the milk reached a temperature of 60°C for the gelatin and carrageenan series and 50°C for the algin preparation series, and then lowered to 170°F.

Time-temperature curves for 15-minute holding period at 77±1°C

The time-temperature curves for the 15-minute holding period at 77±1°C after the addition of gelling agent and sweetener exhibited greater variation. Each graph in Figure 4 presents the mean time-temperature relationships for three gelling agents at one type and concentration of sweetener. Steam kettle temperature was regulated between 170°F and 180°F to maintain the custard mass at 77±1°C.

Although gelling agents presented time-temperature patterns which appeared similar for all sweetener concentrations, the greatest variation occurred within the 40 per cent sucrose series. All treatments exhibited a drop in temperature upon the addition of the gelling agent-sweetener mixture. This rank order of temperature decrease was: carrageenan series > gelatin series > algin preparation series. These differences

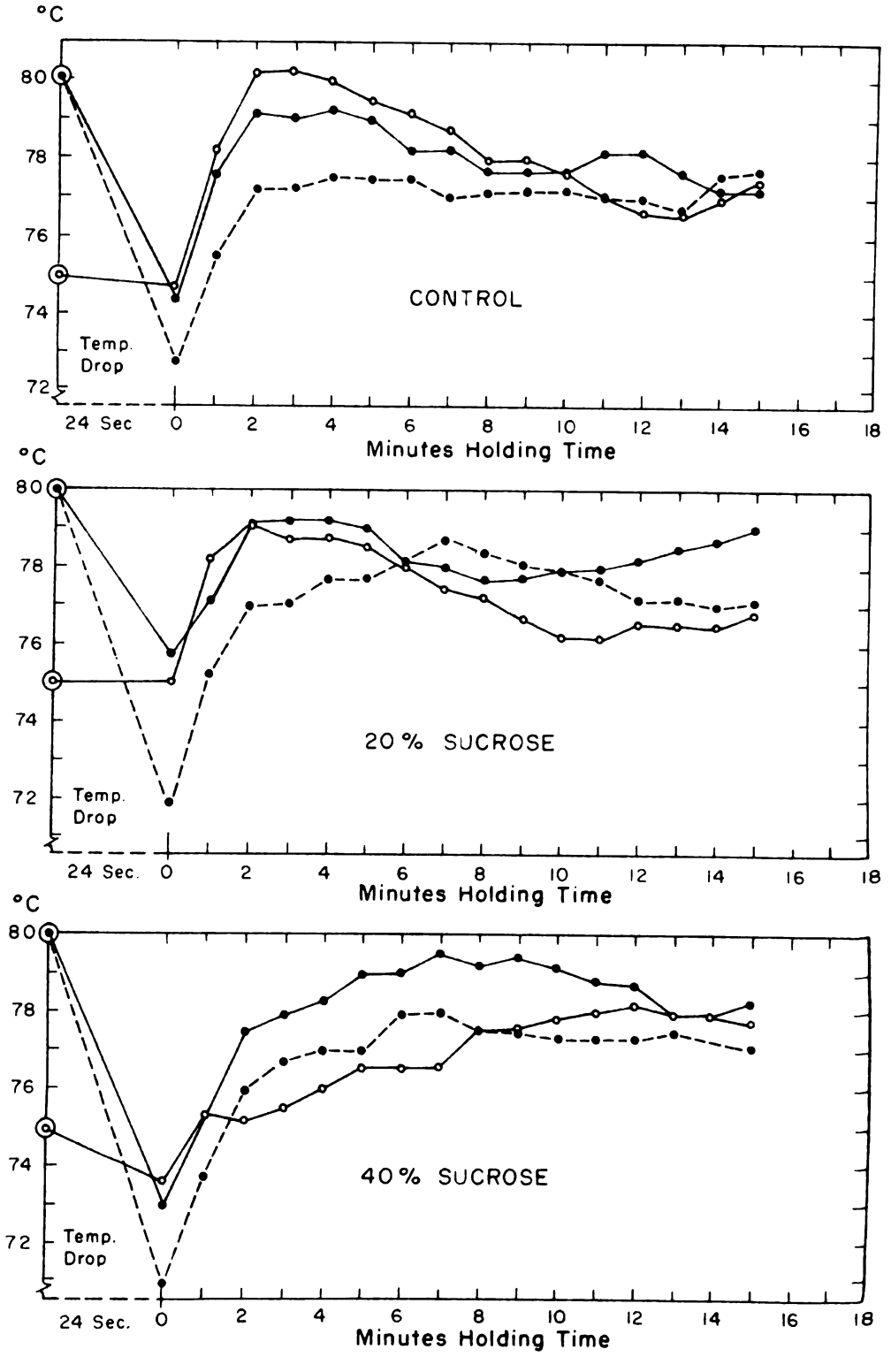

Figure 4. Mean time-temperature relationships for three gelling agents with two concentrations of two types of sweeteners and a control during the 15-minute holding period.

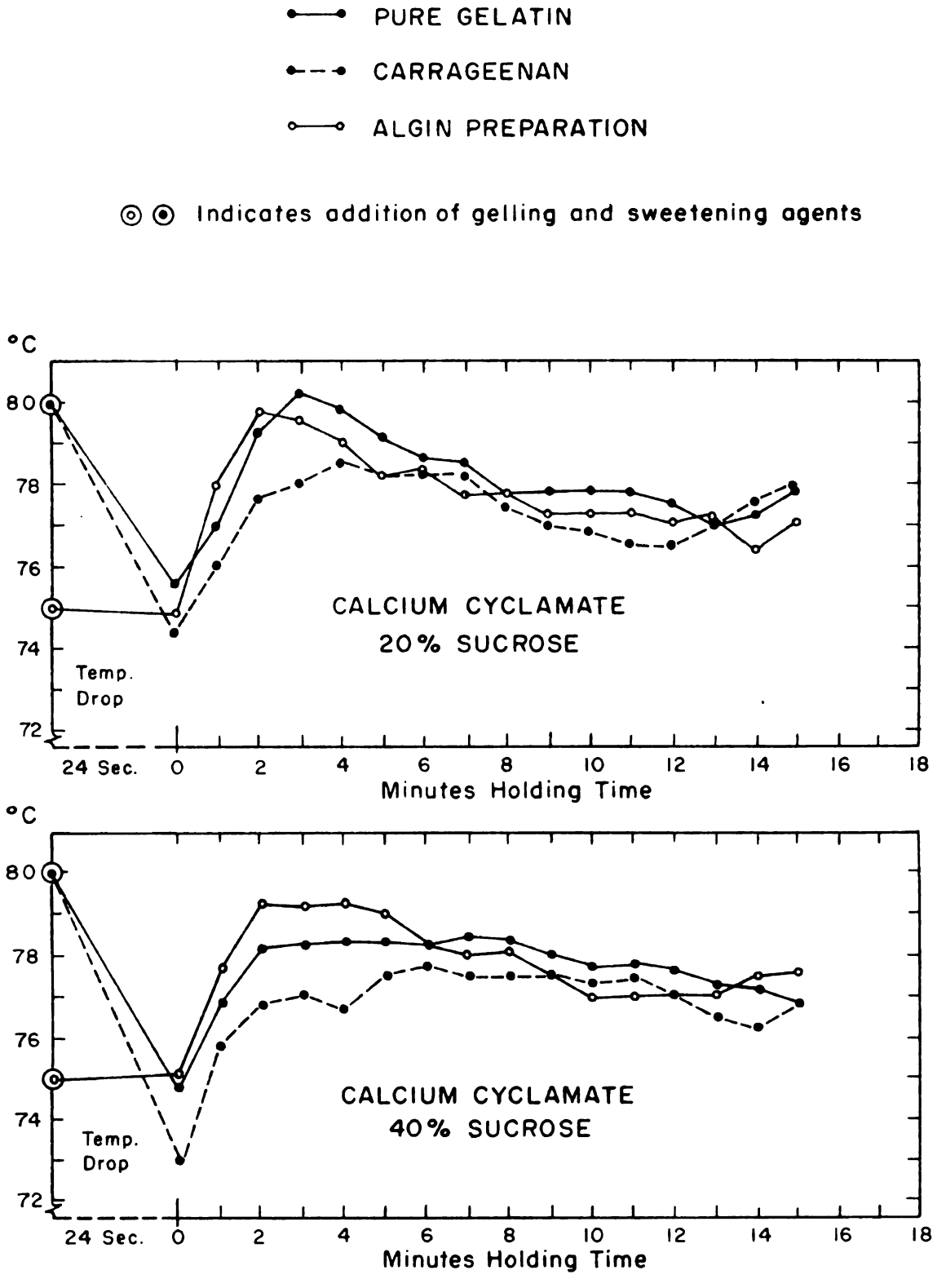

Figure 4. (Continued)

in degree of temperature drop were attributable to specific techniques used for incorporating gelling agents.

Gelatin time-temperature relationships. The gelatin series showed a temperature drop of approximately 5°C after gelling agent-sweetener addition followed by an immediate rise in temperature which peaked between 2 and 5 minutes except for 40 per cent sucrose which peaked between 7 and 9 minutes. This peak was followed by a progressive temperature drop with the last portion of the holding period exhibiting a fairly constant temperature. It appears probable from this pattern that the swelling of gelatin and cornstarch occurs during the last half of the holding period.

Carrageenan time-temperature relationships. Curves for the carrageenan custard samples showed a temperature decrease of 6.5°C followed by a rise in temperature which peaked between 4 and 7 minutes. This peak temperature was lower than peak temperatures of both gelatin and algin preparation samples. Following this, the temperature dropped slightly and then remained fairly constant. The slower rise in temperature may indicate the swelling of carrageenan occurs soon after addition of the gelling agent-sweetener mixture and, as a result, the temperature of the custard mass remains fairly constant. Furthermore, the initial addition of a cooler mixture, as shown by the increased drop in temperature, may also influence the rate of temperature rise.

Algin preparation time-temperature relationships. Time-temperature patterns for the algin preparation series except those sweetened with 40 per cent sucrose exhibited a slight drop of 0.2°C followed by a rise

in temperature which peaked at approximately 2 minutes. This peak was followed by a steady decrease in temperature which ended about 8 minutes later, remained fairly constant, and finally rose just prior to the end of the holding period. This curve appears to indicate that swelling of algin preparation occurred shortly after its addition and the reaction may be more endothermic than the swelling of the other gelling agents thus causing the greatest decrease in temperature. The temperature rise at the end of the holding period may indicate that, under these conditions, the swelling of algin preparation and cornstarch is completed after 13 or 14 minutes.

Custards gelled with algin preparation and sweetened with 40 per cent sucrose showed a slower rise in temperature which peaked at approximately 12 minutes followed by a slight decrease in temperature. This curve did not exhibit a temperature increase at the end of the holding period.

Heating curves after egg-milk mixture addition

The heating curves of the mixture after the addition of the egg-milk mixture varied only as the end temperatures varied. The addition of this mixture caused a 6 to 7°C drop in temperature and the average heating times for end points of 74, 77, and 80°C were 3.2, 4.2, and 5.7 minutes, respectively. The rate of heating was controlled to maintain 5 pounds of pressure with a thermostatic setting of 185°F.

Time-temperature relationships for the 15-minute holding period and for the heating after the addition of the egg-milk mixture show variations in the amount of heat to which the gelling agents were exposed. Gelatin, in solution, undergoes degradation under the influence of heat, especially at extremes of the pH scale. In a study of heat

degradation of gelatin prepared from oxhide, Ames (3) reported the breakdown of gelatin is slowest at neutrality and is independent of concentration. The investigator's results show a 2 per cent solution heated for 2 hours at 85°C at neutrality lost slightly over 1/5 of its original gelling power. The processor (58) reported carrageenan is not sensitive to heat above pH of 3.5 and at pH 7 can be retorted under pressure for several hours. Within the limits of this study, no consistent relationship was found between gel strength and time-temperature relationships during the cooking process.

Gel Strength and Syneresis Tests

Gel strength was measured both as the ability to resist penetration and as the ability to hold a rigid shape. Resistance to penetration was determined by a Precision Universal Penetrometer with a 5-second penetration for a 150-gram load cone attachment. Rigidity of the sample was determined as per cent sag calculated from measurements of the height of free-standing inverted samples before and after a specified time interval. The results were analyzed for effects of gelling agents and two chemically different sweetening agents: sucrose, a non-reducing disaccharide classified as a non-electrolyte, and calcium cyclamate, a strong electrolyte which is 90 per cent ionized in solution. The weight of drainage due to syneresis was determined for a 30-minute period with controlled evaporation. Throughout the discussion of both gel strength tests and the test for syneresis jellied custards prepared without sweetener, 20 per cent sucrose, 40 per cent sucrose, calcium cyclamate = 20 per cent sucrose, and calcium cyclamate = 40 per cent

sucrose will be referred to as sweetener A, B, C, D, and E, respectively.

Penetration

Mean penetration readings, based on four replications per treatment, and conglomerate averages for three gelling agents and five concentrations of sweetener are summarized in Table 5. The original penetrometer readings are presented in the Appendix.

Table 5. Summary of penetrometer values for jellied custard: treatment means and conglomerate averages for gelling and sweetening agents (millimeters).

Sweetening Agent	Gelling Agent: Pure Gelatin	Carrageenan	Algin Preparation	Sweetening Agent Ave.	S.S.R.V.[a]	5%	1%
A	27.48[c]	31.54	26.48	28.49	Treatment	0.70	0.94
B	27.12	30.79	26.09	28.00			
C	27.72	31.16	25.44	28.11	Sweetening		
D	27.34	33.07	29.87	30.09	Agent	0.40	0.54
E	25.69	33.35	33.97	31.00			
G. A. Ave.[b]	27.07	31.98	28.37		Gelling Agent	0.31	0.42

[a]Significant Studentized range values for 2 consecutive averages.
[b]Gelling agent average.
[c]Based on four replications.

Analysis of variance of these data revealed significant differences in gel strength, at the 1 per cent level of probability, attributable to treatments. The Studentized range test was utilized to sort out significant gel strength differences due to gelling agents, sweetening agents, and individual treatments. The rank order of differences are presented in Table 6.

Table 6. Mean squares and rank order of significant differences for penetration of jellied custard.

Source of Variance	D. F.	Mean Square	Significant Differences[a] 1% level	Additional at 5% level
Penetration	14	35.32**	1 > 3 > 2	
			B C A > D > E	B C A
			E_1 > B_1 D_1 A_1 C_1	
			B_2 C_2 A_2 > D_2 E_2	B_2 C_2 A_2
			A_3 B_3 C_3 > D_3 > E_3	
			A_3 A_1 > A_2	A_3 > A_1
			B_3 > B_1 > B_2	
			C_3 > C_1 > C_2	
			D_1 > D_3 > D_2	
			E_1 > E_2 E_3	

**Significant at 1 per cent level of probability.

[a]Significantly greater than those that follow. Treatments are listed in ascending averages. Underlining denotes no significant difference.

Key: 1 Pure gelatin conglomerate average
2 Carrageenan conglomerate average
3 Algin preparation conglomerate average
A Control conglomerate average
B 20% sucrose conglomerate average
C 40% sucrose conglomerate average
D Ca. cyclamate = 20% sucrose conglomerate average
E Ca. cyclamate = 40% sucrose conglomerate average
A_1 B_1 C_1 D_1 E_1; A_2 B_2 C_2 D_2 E_2; A_3 B_3 C_3 D_3 E_3 Treatment unit ave.

Gel strength for gelling and sweetening agents. Conglomerate averages for penetration data rank gel strength for custards prepared with three gelling agents as gelatin > algin preparation > carrageenan custards, significant at 1 per cent level of probability. At the same level of probability, penetrometer values for conglomerate sweetener averages show samples made with the following types and concentrations of sweeteners ranked gel strength as: B = C = A > D > E. Additional significance at 5 per cent probability indicate that custards prepared with B have greater gel strength than custards prepared with A.

Gel strength differences within gelatin series. Samples from the treatment combining gelatin with the highest concentration of calcium cyclamate were significantly stronger, at the 1 per cent level of probability, than samples from treatments combining gelatin with the other type and concentrations of sweetener. Lowe (39) noted that below the isoelectric point of gelatin, cations have more effect than anions. Thus calcium would have more effect than cyclamate at the pH reported for the gelatin custards of the present study. These gel strength findings agree with the findings of Nobel (48) who stated that calcium strengthens gelatin gels. The lack of significance for gel strength of samples with sucrose addition disagrees with the works of Friedman and Shearer (25) and Advani and Narwani (1). It is likely that the effect of sucrose may be offset by the significant increase in custard volume.

Gel strength differences within carrageenan series. When carrageenan was used as the gelling agent, non-sweetened and sucrose-sweetened custards had significantly higher gel strength than cyclamate-sweetened

custards. The B-custard exhibited greater gel strength than the A-sweetened custard at the 5 per cent level of probability. The increased gel strength of samples made with 20 per cent sucrose supports the theory of Tressler and Lemon (68) who proposed that the presence of solutes in colloidal solutions of carrageenan compete with the colloidal micelles for water. Dilution of gelling agent due to the increase in batch yield may explain why gel strength increased significantly only for the lower concentration of sucrose. The effect of calcium cyclamate on carrageenan gels has not been reported in the literature. Possibly increased ionic strength or a specific effect of calcium ions or the large cyclamate anions contributes to the results noted in this study.

Gel strength differences within algin preparation. For samples prepared with algin preparation, the A custard was significantly stronger, 1 per cent level of probability, than C custard. At this same level the A, B, and C custards had greater gel strength than D custard. All the foregoing custards were stronger than the custard prepared with the higher concentration of cyclamate. Scott (57) reported that complexes formed by polyanions such as alginic acid with cationic detergents were soluble in salt solutions at concentrations characteristic to the polyanion polymer and that 0.33 N. potassium chloride or 0.30 N. magnesium chloride had sufficient ionic strength to maintain 70 per cent of the alginate in solution. Although the normalities of calcium cyclamate concentrations used in the study under discussion were approximately 0.02 and 0.05, respectively, and would not appreciably affect ionic strength of the custard formula, calcium cyclamate addition appeared to reduce gel strength.

Gel strength differences within sweetener series. Custards prepared with the three gelling agents for A, B, and C sweeteners ranked gel strength as follows: algin preparation > gelatin > carrageenan custard. For samples prepared with D sweetener, the gel strength of the algin preparation and gelatin custards was reversed. The rank order of gel strength for E-sweetened custards was gelatin > algin preparation > carrageenan samples.

The inverted samples in Figure 5, representing 15 treatments, do not illustrate as great a variation in gel strength as is indicated by significant differences in mean penetrometer measurements. Differences in inverted heights are readily observed in the cyclamate series of custards. As can be seen in Table 5, page 58, samples prepared with calcium cyclamate = 40 per cent sucrose exhibited the greatest variation in millimeters of penetration for treatment means, ranging from 25.69 to 33.97 mm.

Per cent sag

Original per cent sag data appear in the Appendix. From this table it can be seen that sag percentages for the treatment combining algin preparation and sweetener E were at least 5 times greater than sag percentages of all other treatments. This treatment was significantly different from all others and these values were not included in the analysis of variance. Mean per cent sag values for fourteen treatments, based on four replications per treatment, and conglomerate averages for three gelling agents and five concentrations of sweetener are summarized in Table 7.

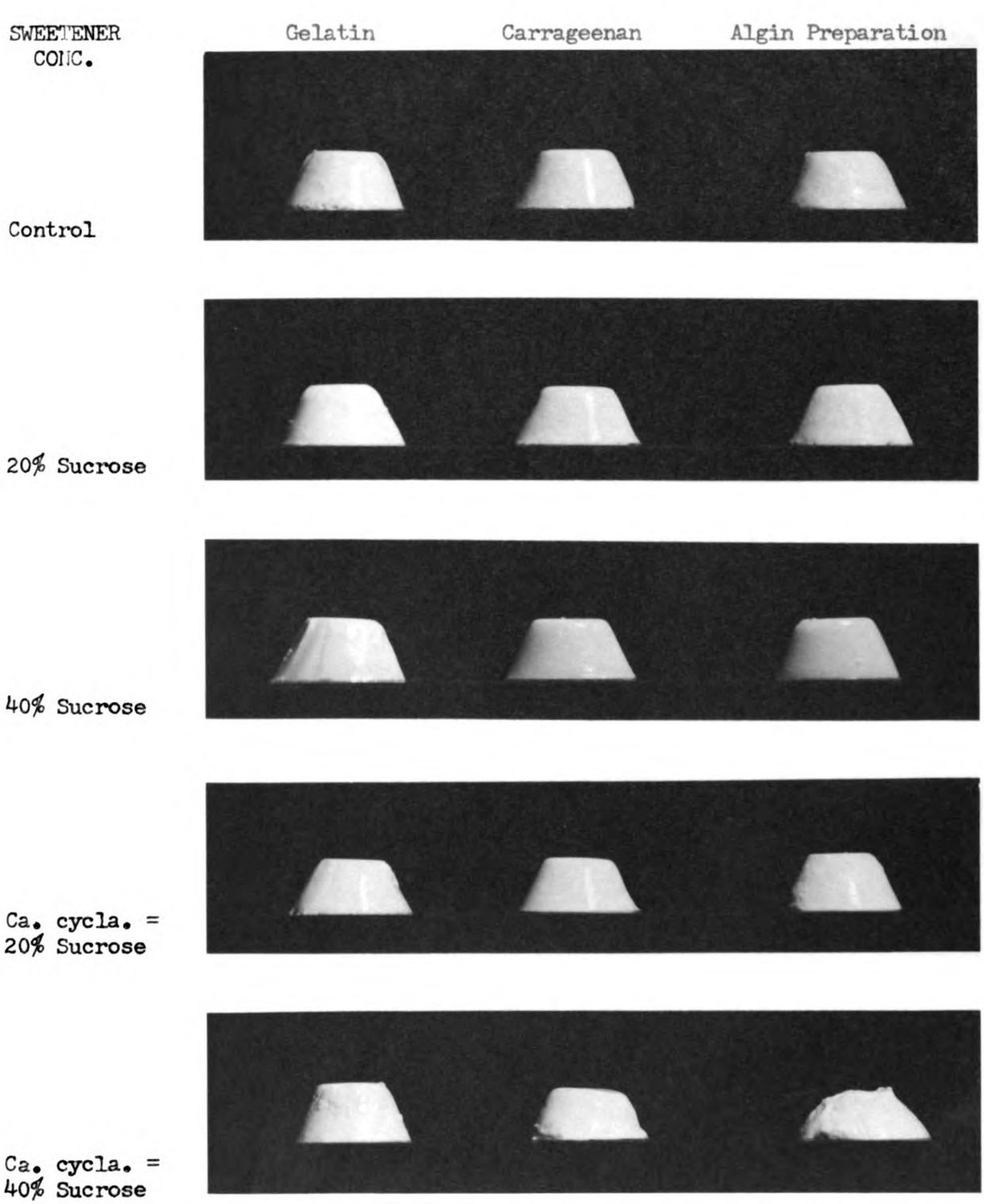

Figure 5. Initial inverted heights of jellied custards prepared with three gelling agents and five concentrations of sweetener.

Table 7. Summary of per cent sag values for jellied custard: treatment means and conglomerate averages for gelling and sweetening agents.

Sweetening Agent	Gelling Agent: Pure Gelatin	Gelling Agent: Carrageenan	Gelling Agent: Algin Preparation	Sweetening Agent Ave.	S.S.R.V.[a]	5%	1%
A	2.81[c]	2.99	0.87	2.22	Treatment	0.66	0.88
B	2.79	3.68	1.88	2.79			
C	2.85	4.70	1.34	2.96	Sweetening		
D	1.92	5.98	3.32	3.74	Agent	0.38	0.51
E	1.72	5.11	---	3.41			
G.A. Ave.[b]	2.42	4.49	1.85		Gelling Agent	0.29	0.39

[a]Significant Studentized range values for 2 consecutive averages.
[b]Gelling agent averages.
[c]Based on four replications.

Analysis of variance of these data revealed significant differences in rigidity among treatments, at the 1 per cent level of probability. The Studentized range test was used to establish significant rigidity differences due to gelling agents, sweetening agents, and individual treatments. The rank order of these differences is presented in Table 8.

Rigidity differences for gelling and sweetening agents. The conglomerate averages of gelling agents rank custard rigidity as algin preparation > gelatin > carrageenan custard. In comparison with penetration findings, algin preparation and gelatin custards have reversed positions. This reversal may result from the omission of data for one algin preparation treatment.

Conglomerate averages of sweetening agents indicate the non-sweetened samples are significantly more rigid than the other types and concentrations of sweeteners, at a probability of 1 per cent. At the

Table 8. Mean squares and rank order of significant differences for per cent sag of jellied custard.

Source of Variance	D. F.	Mean Square	Significant Differences[a] 1% level	Additional at 5% level
Per cent Sag	13[b]	113.09**	3 > 1 > 2	
			A > B C E D	B C > E D
			E_1 D_1 B_1 A_1 C_1	E_1 D_1 > B_1 A_1 C_1
			A_2 > B_2 > C_2 E_2 D_2	C_2 E_2 > D_2
			A_3 C_3 B_3 > D_3	A_3 > C_3 B_3
			A_3 > A_1 A_2	
			B_3 B_1 B_2	
			C_3 > C_1 > C_2	
			D_1 > D_3 > D_2	
			E_1 > E_2	

**Significant at 1 per cent level of probability.

[a]Significantly greater than those that follow. Treatments are listed in ascending averages. Underlining denotes no significant difference.

[b]Algin preparation---Ca. cyclamate = 40% sucrose was not included.

Key: 1 Pure gelatin conglomerate average
2 Carrageenan conglomerate average
3 Algin preparation conglomerate average
A Control conglomerate average
B 20% sucrose conglomerate average
C 40% sucrose conglomerate average
D Ca. cyclamate = 20% sucrose conglomerate average
E Ca. cyclamate = 40% sucrose conglomerate average
A_1 B_1 C_1 D_1 E_1; A_2 B_2 C_2 D_2 E_2; A_3 B_3 C_3 D_3 E_3 Treatment unit ave.

5 per cent level, samples containing 20 and 40 per cent sucrose are significantly more rigid than samples containing the two concentrations of calcium cyclamate.

Rigidity differences for gelatin series. When gelatin was used as the gelling agent, samples prepared with sweetener E were significantly more rigid, at the 1 per cent level of probability, than samples prepared with sweeteners B, A, and C. Within this series mean sag percentages of E and D custards as well as D, B, A, and C custards were not significantly different. However, custards containing either concentration of cyclamate were more rigid than non-sweetened and sucrose-sweetened custards at 5 per cent level of probability. Since rigidity and gel strength are closely related, these results are also in accord with the findings of Nobel (48).

Rigidity differences for carrageenan series. Samples made with carrageenan and various sweetener concentrations rank in rigidity, significant at 1 per cent probability, as A > B > C = E but > D and E = D-custards. At the 5 per cent probability, custards made with the higher concentration of calcium cyclamate were more rigid than custards prepared with the lower concentration of calcium cyclamate. These findings disagree with Tressler and Lemon's (68) theory that the presence of solutes decrease free water thus allowing less liquid for the gelling agent to entrain. Elasticity has been noted in the manufacturer's bulletin (58) as characteristic for sucrose-carrageenan gels.

Rigidity differences for algin preparation series. Significant differences in rigidity for custards prepared with algin preparation show samples sweetened with cyclamate had lower rigidity than non-sweetened and sucrose-sweetened samples. Custards prepared with the higher concentration of calcium cyclamate formed a very tender gel structure. Possibly the calcium alginate is dissolved by the salt concentration as has been reported by Scott (57); however in view of the normalities of calcium cyclamate used in this study, this explanation appears questionable. These findings evoke two important questions: When present in relatively small concentrations, does calcium cyclamate affect the gel structure of alginates? What percentage of soluble alginate must be reached before the gel formation of the system is adversely affected?

Rigidity differences within sweetener series. Non-sweetened custards ranked in rigidity as algin preparation > gelatin = carrageenan samples. Algin preparation and gelatin custards sweetened with 20 per cent sucrose as well as gelatin and carrageenan samples sweetened with 20 per cent sucrose showed no significant difference in rigidity. Forty per cent sucrose-sweetened custards exhibited the rank order of algin preparation > gelatin > carrageenan samples for rigidity. When cyclamate = 20 per cent sucrose was the sweetening agent, rigidity was greatest for gelatin custards and lowest for carrageenan samples. Gelatin custards sweetened with the higher level of calcium cyclamate were more rigid than carrageenan custards sweetened with the higher level of calcium cyclamate.

Figure 5, page 63, illustrates the initial inverted height of samples representing the 15 treatments. From this, the per cent sag of custards containing cyclamate can be observed. Tenderness of gel

structure for the custard representing the treatment combining of algin preparation with sweetener E may be noted from the visible degree of sag.

Syneresis

Table 9 summarizes mean drainage values, based on four replications per treatment, and conglomerate averages for three gelling agents and five concentrations of sweetener. Original syneresis values are reported in the Appendix.

Table 9. Summary of drainage values for jellied custard: treatment means and conglomerate averages for gelling and sweetening agents (grams).

Sweetening Agent	Gelling Agent: Pure Gelatin	Carrageenan	Algin Preparation	Sweetening Agent Ave.	S.S.R.V.[a]	5%	1%
A	0.08[c]	1.00	0.50	0.53	Treatment	0.65	0.87
B	0.07	0.75	0.34	0.39			
C	0.45	1.01	0.36	0.60	Sweetening Agent	0.32	0.44
D	0.12	2.21	0.20	0.84			
E	0.11	5.27	0.33	1.90			
G. A. Ave.[b]	0.16	2.05	0.34		Gelling Agent	0.29	0.39

[a]Significant Studentized range values for 2 consecutive averages.
[b]Gelling agent averages.
[c]Based on four replications.

Analysis of variance of drainage due to syneresis revealed that there were significant differences attributable to treatment, at the 1 per cent level of probability. Significant drainage differences due to gelling agents, sweetening agents, and individual treatments were found by use of the Studentized range test. The rank order of these data is presented in Table 10.

Table 10. Mean squares and rank order of significant differences for drainage of jellied custard.

Source of Variance	D. F.	Mean Square	Significant Differences[a] 1% level	Additional at 5% level
Syneresis	14	7.21**	2 > 3 1	
			E > D C A B	D C A B
			E_2 > D_2 > C_2 A_2 B_2	
			D_2 > D_3 D_1	
			E_2 > E_3 E_1	

**Significant at 1 per cent level of probability.

[a]Significantly greater than those that follow. Treatments are listed in descending averages. Underlining denotes no significant difference.

Key: 1 Pure gelatin conglomerate average
2 Carrageenan conglomerate average
3 Algin preparation conglomerate average
A Control conglomerate average
B 20% sucrose conglomerate average
C 40% sucrose conglomerate average
D Ca. cyclamate = 20% sucrose conglomerate average
E Ca. cyclamate = 40% sucrose conglomerate average
A_1 B_1 C_1 D_1 E_1; A_2 B_2 C_2 D_2 E_2; A_3 B_3 C_3 D_3 E_3 Treatment unit ave.

Drainage differences for gelling and sweetening agents. Drainage due to syneresis was significantly greater for samples with carrageenan used as the gelling agent than for samples with gelatin or algin preparation used as the gelling agents. Conglomerate averages for sweeteners show that custards containing sweetener E exhibited a larger weight of

drainage than all others, significant at 1 per cent probability. At 5 per cent probability custards sweetened with the lower concentration of calcium cyclamate had a larger weight of drainage than custards sweetened with 20 per cent sucrose.

Drainage differences within gelatin and algin preparation series. No significant differences for drainage were found between custards gelled with gelatin or algin preparation.

Drainage differences within carrageenan series. For samples gelled by carrageenan, the weight of drainage varied with sweetener addition, significant at 1 per cent probability: E > D > C = A = B samples.

Drainage differences within sweetener series. Sucrose- and non-sweetened custards exhibited no significant differences in the weight of drainage due to syneresis when combined with a particular gelling agent. For both concentrations of cyclamate the amount of drainage from custards prepared with carrageenan was significantly greater, 1 per cent probability, than custards made with algin preparation or gelatin. The weight of drainage from custards sweetened with cyclamate and gelled with algin preparation or gelatin was not significantly different.

Texture of Non-sweetened Jellied Custard

Texture differences of non-sweetened jellied custard prepared with gelatin, carrageenan, and algin preparation were subjectively compared both by mouth-feel and by appearance of the cut sample. The texture of samples, noted as mouth-feel by the investigator, may be described as

follows: gelatin -- smooth, clings to the roof of the mouth; carrageenan -- extremely smooth, airy, melts in the mouth; and algin preparation -- pasty, rough, almost gritty.

Differences in cut surface appearance of non-sweetened custards are illustrated in Figure 6. Texture of the gelatin custard is neither extremely smooth nor coarse. Small air bubbles are apparent in the otherwise smooth carrageenan sample. The custard containing algin preparation exhibits a coarse, pebbly texture. The gelatin and the carrageenan custards display a bubbly surface whereas the surface of the algin preparation sample is quite smooth.

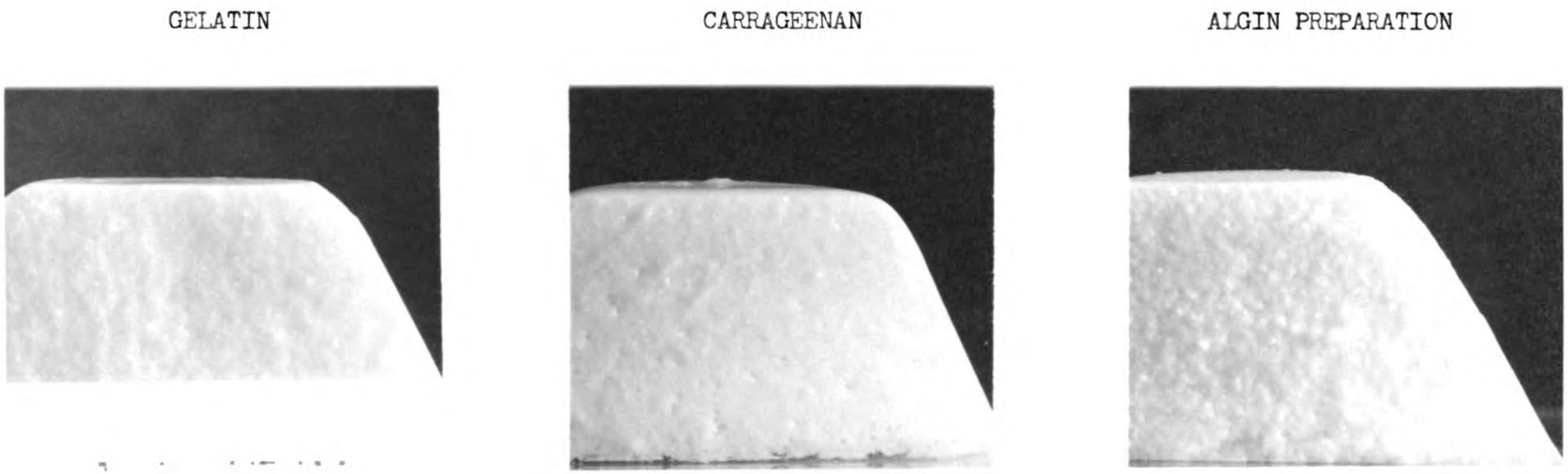

Figure 6. Cut surfaces of non-sweetened jellied custard prepared with three gelling agents.

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to compare the effects of two concentrations of sucrose and two concentrations of calcium cyclamate upon the gel strength of selected gelling agents in the preparation of jellied custard. The gelling agents, arbitrarily chosen from products recommended by their respective manufacturers for use in milk-type desserts, were acid-processed, pure, unflavored gelatin with a bloom value of 220; carrageenan;[1] and algin preparation.[2]

The basic experimental formula consisted of constant proportions of whole milk solids, whole egg, cornstarch, salt, and water. This formula was modified for series 1, 2, and 3 by the addition of pure gelatin, carrageenan, and algin preparation in the gram-ratio of 1.00 : 0.179 : 0.857, respectively. This ratio was established through preliminary laboratory investigation of equivalent gel strength among agents for a standard milk-cornstarch system. Each series was further modified by the type and concentration of sweetener added: 20 and 40 per cent sucrose, based on total weight of the modified formula exclusive of water; dry calcium cyclamate in amounts required for sweetness comparable to the sucrose concentrations selected for the study; and a control without sweetener for each gelling agent.

All products were cooked in a 10-quart Groen Steam Kettle. Preliminary preparation, cooking procedure, and sample preparation were

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[1]Type 10 Carrageenan manufactured by Marine Colloids, Inc., Chicago 5, Ill.

[2]Margel, a mixture of algin and calcium carbonate, manufactured by Kelco Company, Chicago 6, Ill.

kept as similar as feasible. Differences among suitable dispersion methods for the selected gelling agents necessitated slight procedural variations. All samples were refrigerated at 3.3 to 4.4°C for 19.3 to 19.6 hours before objective evaluation.

Data relative to water hardness, relative humidity of the laboratory during preparation, time and temperature of refrigerated storage, pH of raw egg and jellied custard samples, and batch yield were collected. Time-temperature relationships during cooking were recorded on a Minneapolis-Honeywell Multipoint Recorder.

Objective tests were made to determine gel strength, measured both as the ability to resist penetration and as the ability to hold a rigid shape, and syneresis. Resistance to penetration was determined by a Precision Universal Penetrometer with a 5-second penetration for a 150-gram load cone attachment. Measurements for the calculation of per cent sag were taken to compare the rigidity of custards. Per cent sag was calculated from the difference between the inverted sample height before and after a 15-minute free-standing period divided by the initial inverted sample height determination. Drainage due to syneresis of a free-standing inverted sample was measured as the weight of the drainage resulting from a 30-minute standing period with controlled evaporation.

Water hardness, physical factors, and pH data indicated only minimal variation. Variance resulting from the addition of types and concentrations of sweeteners was found to be significant for batch yield. The orderly relationship between weight of sweetener and batch yield appeared to account for volume increases of all custards sweetened with sucrose, all custards sweetened with the lower concentration of calcium

cyclamate, and algin preparation custards sweetened with the higher concentration of calcium cyclamate. Mean batch yields for gelatin or carrageenan samples sweetened with the higher concentration of calcium cyclamate exhibited smaller volumes than samples made with these gelling agents and sweetened with the lower concentration of calcium cyclamate. This reversal suggests the higher cyclamate concentration may have affected the gelling capacity of these two agents. Furthermore, since differences in total weight of ingredients used affect the dilution of the gelling agent, it is conceivable that gel strength differences apparent in this study may be due, in part, to the variation in batch yield.

Heating curves of milk and of the custard after the addition of the egg-milk mixture showed only slight variation among treatments. Within the limits of the study, time-temperature relationships for the 15-minute holding period at 77±1°C established consistent patterns for each gelling agent at all concentrations of sweetener. Time-temperature cooking curves also presented thermal exposure during the holding period for each treatment. No consistent relationship was found between gel strength of the sample and time-temperature relationships during cooking.

Significant differences for gel strength were established from both penetration and per cent sag data. Custards gelled with algin preparation or gelatin exhibited greater gel strength than custards gelled with carrageenan. The rank order for gelatin and algin preparation custards varied with the type and concentration of sweetener present. Sucrose-sweetened custards gelled with algin preparation seemed to have greater gel strength than sucrose-sweetened custards gelled with gelatin whereas an inverse gel strength relationship occurred for cyclamate-sweetened samples gelled with these two agents.

Per cent sag data showed rigidity of carrageenan gels decreased with sucrose addition and this effect was greater as the concentration of sucrose increased. Penetration data, however, did not support these findings.

The addition of dry calcium cyclamate seemed to increase gel strength for gelatin custards while it decreased gel strength for both carrageenan and algin preparation custards. This effect was increased as the concentration of calcium cyclamate was increased. Custards gelled with algin preparation and sweetened with the higher concentration of calcium cyclamate exhibited the largest decrease in rigidity.

Variance in the weight of drainage due to syneresis among treatments was found to be significant. Syneresis was evident only for custards gelled with carrageenan and the weight of drainage appeared to be enhanced by the addition of dry calcium cyclamate.

Custard texture for non-sweetened samples gelled with gelatin, carrageenan, and algin preparation can be described as moderately smooth, extremely smooth and airy, and coarse and pebbly, respectively. Gelatin and carrageenan custards displayed a bubbly surface whereas the surface of algin preparation custards was quite smooth.

Although the jellied custards were not evaluated for flavor, all custards except those sweetened with the higher concentration of calcium cyclamate were considered acceptable by the investigator. Custards prepared with calcium cyclamate = 40 per cent sucrose had a bitter aftertaste.

The findings of this limited study indicate custard gel strength is a function of both gelling agent and type and concentration of

sweetener used. Evaluation of these findings evoked certain questions which suggest the need for study in these related areas: (1) a study in which the amount of gelling agent is equilibrated for increased volume contributed by the sweetening agent to determine whether dilution of the gelling agent has contributed significantly to the findings of the current study; (2) sodium cyclamate should be substituted for calcium cyclamate to determine whether the effect of dry calcium cyclamate on gel strength was due to calcium or cyclamate ions; and (3) varied amounts of gelling agents should be used in custards sweetened with calcium cyclamate to determine whether the effect of calcium cyclamate on gel strength could be overcome.

Further investigations are warranted to establish whether syneresis is inherent in the milk-type carrageenan gel or whether formula modifications can overcome this drainage.

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APPENDIX

Table 11. Mean penetrometer values for gel strength equilibration (millimeters).

Replication	Gelling Agents		
	Pure Gelatin (14 gm.)	Carrageenan (2.5 gm.)	Algin Preparation (12 gm.)
1	27.20[a]	26.60	28.17
2	27.47	26.23	27.00
3	29.27	28.00	25.27
4	23.43	25.37	25.57
5	25.70	24.63	26.23
6	24.67	25.97	25.93
Average	26.31	26.27	26.36

[a]Based on three readings.

Table 12. Analysis of variance of mean penetrometer values for gel strength equilibration.

Source of Variance	Degrees of Freedom	Sums of Squares	Mean Squares
Total	17	44.59	
Gelling agents	2	0.17	0.09
Error	15	44.42	2.96

Table 13. Modified experimental formulas used in the preparation of jellied custard.

Treatment	Ingredients									
	Whole milk solids (gm.)	Water (ml.)	Whole eggs (gm.)	Corn-starch (gm.)	Salt (gm.)	Sucrose (gm.)	Calcium cyclamate (gm.)	Pure gelatin (gm.)	Carrageenan (gm.)	Algin preparation (gm.)
1-A	510.4	3784	486	54	6.0	---	---	56	---	---
1-B	510.4	3784	486	54	6.0	275	---	56	---	---
1-C	510.4	3784	486	54	6.0	735	---	56	---	---
1-D	510.4	3784	486	54	6.0	---	9.17	56	---	---
1-E	510.4	3784	486	54	6.0	---	24.5	56	---	---
2-A	510.4	3784	486	54	6.0	---	---	---	10	---
2-B	510.4	3784	486	54	6.0	275	---	---	10	---
2-C	510.4	3784	486	54	6.0	735	---	---	10	---
2-D	510.4	3784	486	54	6.0	---	9.17	---	10	---
2-E	510.4	3784	486	54	6.0	---	24.5	---	10	---
3-A	510.4	3784	486	54	6.0	---	---	---	---	48
3-B	510.4	3784	486	54	6.0	275	---	---	---	48
3-C	510.4	3784	486	54	6.0	735	---	---	---	48
3-D	510.4	3784	486	54	6.0	---	9.17	---	---	48
3-E	510.4	3784	486	54	6.0	---	24.5	---	---	48

Key: 1 - Gelatin 2 - Carrageenan 3 - Algin preparation A - Control B - 20% Sucrose
C - 40% Sucrose D - Calcium cyclamate = 20% Sucrose E - Calcium cyclamate = 40% Sucrose

Table 14. Water hardness, physical factors, and pH for jellied custard made with three gelling agents and two concentrations of two sweeteners.[a]

Treatment	Water Hardness (ppm)	Physical Factors			pH Range	
		Relative Humidity	Hours Stored	Temp. Stored °C	Raw Egg	Jellied Custard
1-A	312.1	54.0	19.3	3.3	7.1-7.4	6.8-6.9
1-B	307.8	62.5	19.3	3.4	7.1-7.4	6.8-6.9
1-C	299.3	64.0	19.3	3.4	7.1-7.5	6.8-6.9
1-D	303.5	52.0	19.3	3.3	7.1-7.4	6.6-6.7
1-E	307.8	63.9	19.4	3.7	7.1-7.4	6.4-6.6
2-A	312.1	54.8	19.3	4.4	7.2-7.5	7.0-7.0
2-B	307.8	61.7	19.4	3.8	7.1-7.5	7.0-7.0
2-C	299.3	64.2	19.5	4.2	7.3-7.5	6.9-7.1
2-D	303.5	50.2	19.4	4.0	7.1-7.4	6.8-6.9
2-E	307.8	60.0	19.5	4.0	7.3-7.6	6.6-6.7
3-A	312.1	54.8	19.5	3.5	7.1-7.3	7.2-7.3
3-B	307.8	62.0	19.4	3.4	7.1-7.5	7.2-7.3
3-C	299.3	60.2	19.5	3.5	7.1-7.5	7.2-7.3
3-D	303.5	50.0	19.4	3.5	7.3-7.5	7.1-7.2
3-E	307.8	59.2	19.6	3.3	7.1-7.5	6.9-7.0

[a]Values based on four replications per treatment.

Key: 1 - Pure Gelatin
2 - Carrageenan
3 - Algin Preparation

A - Control
B - 20% Sucrose
C - 40% Sucrose
D - Ca. cyclamate = 20% Sucrose
E - Ca. cyclamate = 40% Sucrose

Table 15. Penetrometer values for jellied custard: original data, treatment means, and conglomerate averages for gelling and sweetening agents (millimeters).

Sweetening Agent Type and Concentration	Gelling Agent Series 1 Pure Gelatin	Series 2 Carrageenan	Series 3 Algin Preparation	Sweetening Agent Ave.
A Control	27.33	31.17	26.67	
	27.20	31.10	25.70	
	27.83	32.00	26.57	
	27.56	31.87	26.93	
Mean	27.48	31.54	26.48	28.49
B 20% Sucrose	26.63	30.77	25.63	
	27.20	30.23	26.63	
	27.57	31.03	25.53	
	27.47	31.13	26.57	
Mean	27.12	30.79	26.09	28.00
C 40% Sucrose	27.30	31.07	24.80	
	28.13	31.47	26.00	
	27.43	31.47	25.80	
	28.03	30.63	25.17	
Mean	27.72	31.16	25.44	28.11
D Ca. Cyclamate = 20% Suc.	27.07	32.70	30.93	
	27.30	33.27	29.77	
	27.17	33.07	29.50	
	27.83	33.23	29.27	
Mean	27.34	33.07	29.87	30.09
E Ca. Cyclamate = 40% Suc.	24.67	32.97	34.23	
	26.23	33.53	33.53	
	25.73	33.43	34.43	
	26.13	33.47	33.67	
Mean	25.69	33.35	33.97	31.00
Gelling Agent Average	27.07	31.98	28.37	

Significant Studentized Range Value for 2 Consecutive Averages	5% level	1% level
Treatment means	0.70	0.94
Sweetening agent averages	0.40	0.54
Gelling agent averages	0.31	0.42

Table 16. Per cent sag values for jellied custard: original data, treatment means, and conglomerate averages for gelling and sweetening agents.

Sweetening Agent Type and Concentration	Gelling Agent Series 1 Pure Gelatin	Series 2 Carrageenan	Series 3 Algin Preparation	Sweetening Agent Ave.
A Control	3.56	2.77	0.70	
	2.82	2.82	1.38	
	2.76	2.82	0.69	
	2.11	3.56	0.70	
Mean	2.81	2.99	0.87	2.22
B 20% Sucrose	2.78	4.27	2.07	
	2.81	4.18	2.07	
	2.82	3.51	2.04	
	2.76	2.77	1.34	
Mean	2.79	3.68	1.88	2.79
C 40% Sucrose	2.91	4.84	0.69	
	2.84	4.94	1.38	
	2.76	4.90	1.99	
	2.88	4.21	1.30	
Mean	2.85	4.70	1.34	2.96
D Ca. cyclamate = 20% Suc.	2.05	5.74	4.36	
	1.45	6.58	2.70	
	2.05	5.77	3.47	
	2.13	5.84	2.74	
Mean	1.92	5.98	3.32	3.74
E Ca. cyclamate = 40% Suc.	1.36	5.02	31.36[a]	
	2.12	5.23	27.54[a]	
	1.36	5.12	34.31[a]	
	2.02	5.07	22.85[a]	
Mean	1.72	5.11	29.02[a]	3.41
Gelling Agent Average	2.42	4.49	1.85	

Significant Studentized Range Values for Two Consecutive Averages	5% level	1% level
Treatment means	0.66	0.88
Sweetening agent averages	0.38	0.51
Gelling agent averages	0.29	0.39

[a]Statistician considered data too large to be included in analysis with remaining figures.

Table 17. Drainage values for jellied custard: original data, treatment means, and conglomerate averages for gelling and sweetening agents (grams).

Sweetening Agent Type and Concentration	Gelling Agent			Sweetening Agent Ave.
	Series 1 Pure Gelatin	Series 2 Carrageenan	Series 3 Algin Preparation	
A Control	0.06	1.08	0.48	
	0.09	0.99	0.67	
	0.11	1.02	0.41	
	0.05	0.92	0.45	
Mean	0.08	1.00	0.50	0.53
B 20% Sucrose	0.04	0.82	0.46	
	0.05	0.67	0.53	
	0.06	0.67	0.17	
	0.13	0.84	0.20	
Mean	0.07	0.75	0.34	0.39
C 40% Sucrose	0.71	0.93	0.27	
	0.43	0.86	0.59	
	0.25	1.00	0.20	
	0.39	1.25	0.36	
Mean	0.45	1.01	0.36	0.60
D Ca. cyclamate = 20% Suc.	0.20	3.09	0.06	
	0.10	2.04	0.21	
	0.09	1.38	0.15	
	0.08	2.33	0.36	
Mean	0.12	2.21	0.20	0.84
E Ca. cyclamate = 40% Suc.	0.19	5.11	0.46	
	0.09	7.47	0.47	
	0.06	4.67	0.26	
	0.08	3.83	0.13	
Mean	0.11	5.27	0.33	1.90
Gelling Agent Average	0.16	2.05	0.34	

Significant Studentized Range Values for Two Consecutive Averages	5% level	1% level
Treatment means	0.65	0.87
Sweetening agent averages	0.32	0.44
Gelling agent averages	0.29	0.39