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Isolation and characterization of the rhealogical properties of the gum produced by Alcalgines viscolactis as grown in whey substrate

Joanne C. Harrison

To the Graduate Council:

I am submitting herewith a thesis written by Joanne C. Harrison entitled "Isolation and characterization of the rhealogical properties of the gum produced by Alcalgines viscolactis as grown in whey substrate." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Hugh O. Jaynes, Major Professor

We have read this thesis and recommend its acceptance:

Ada Marie Campbell, W. W. Overcast

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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I am submitting herewith a thesis written by Joanne C. Harrison entitled "Isolation and Characterization of the Rheological Properties of the Gum Produced by Alcaligenes viscolactis as Grown in Whey Substrate." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

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Hilton A. Smith
Vice Chancellor
Graduate Studies and Research

ISOLATION AND CHARACTERIZATION OF THE RHEOLOGICAL PROPERTIES OF THE GUM PRODUCED BY *ALCALIGENES VISCOLACTIS* AS GROWN IN WHEY SUBSTRATE

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Joanne C. Harrison

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ABSTRACT

The objectives of this study were to isolate the gum produced by Alcaligenes viscolactis grown in whey and to characterize the rheological properties of the gum as they pertain to food systems.

A 5% inoculum of A. viscolactis was grown in whey for 120 hours at 21° C. The gum was harvested by acid precipitation followed by washing with water and acetone and air-drying.

The effects of gum concentration, salt concentration, pH, heating treatment, and temperature of measurement on the viscosity of aqueous solutions of the gum were studied. Gum concentration was found to have the largest significant effect on viscosity followed by temperature of measurement, heating treatment, and salt. The effect of pH was not significant. Using the analysis of variance data, a regression polynomial was derived. Correlation between predicted and observed viscosities was 0.91.

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CHAPTER I

INTRODUCTION

Although plant hydrocolloids have traditionally been used for a wide variety of functions in foods and food processes, a newly developing source of hydrocolloids for use in food systems has been found in the biosynthetic capabilities of non-pathogenic microorganisms. These microorganisms produce abundant amounts of gums which are freely dispersed in the culture fluid. The properties of these gums appear to lend themselves to practical utility in the food industry, and thus there has been a growing interest in this new source of rheological modifiers.

Alcaligenes viscolactis is a non-pathogenic microorganism that is known throughout the dairy industry for its ability to produce ropiness in milk. This ropy condition is the result of the presence of an extracellular gum that is produced by the microorganism as it utilizes the milk nutrients for its growth.

The increased viscosity as well as the gummy and stringy appearance of ropy milk might indicate some practical utility for this microbial gum in the food industry. And yet, it becomes evident that using milk for the production of the gum in a large scale operation would not be economically feasible.

However, whey, a by-product of the dairy industry, would be both a readily available and inexpensive substrate. Environmental problems in whey disposal necessitate utilization of this material which is rich in both carbohydrate and protein.

Thus this study was designed to isolate the gum produced by A. viscolactis when grown in whey and to investigate the rheological properties of the microbial hydrocolloid as they pertain to food systems.

CHAPTER II

LITERATURE REVIEW

I. NATURE AND OCCURRENCE OF MICROBIAL GUMS

The ability of microorganisms to produce extracellular gums from simpler organic substances is widespread. These gums are generally in the form of polysaccharides and can be classified into two forms: 1) capsules that are integral with the cell wall and 2) slimes that accumulate outside the cell wall and diffuse constantly into the culture medium (27).

Microbial polysaccharides take on one of two general structures. Homopolysaccharides are composed of only one kind of polymeric unit, whereas heteropolysaccharides contain several kinds of polymeric units (21).

A major factor determining the occurrence in nature of extracellular microbial gums is the availability of carbon-containing substrate material, such as carbohydrates or hydrocarbons under conditions that permit microbial growth (21). However, also of importance is the carbon substrate concentration, the presence of a nitrogen source, temperature, pH, and any mineral requirements of the microorganism (27).

II. RHEOLOGICAL PROPERTIES OF MICROBIAL GUMS

Aqueous solutions of microbial gums are generally described as being viscous, and yet this property is dependent on polysaccharide concentration, temperature, and pH. Likewise, effects of salts and heating may also influence viscosity. Among microbial polysaccharides, differences may exist in the influence of the factors, although patterns of similarity too are evident.

The relation between viscosity and concentration of several microbial gums is shown in Figure 1 (21). The viscosity of dextran, an alpha-D-glucan from Leuconostoc mesenteroides, sharply contrasts that of the heteropolysaccharides B-1459 (xanthan gum from Xanthamonas campestris) and B-1973 (from Arthrobacter viscosus). The dextran solution showed practically no viscosity increase until a concentration of 3% is reached, whereas B-1459 and B-1973 showed high viscosities at relatively low (1%) concentrations.

An often stated characteristic of polyelectrolytes is the sharp decrease in the viscosity of their aqueous solutions upon introduction of an electrolyte in the form of a salt (21). However, a number of the microbial gums do not have this property and are likely to give the opposite response, or display it to a lesser degree. Critical variables in such behavior are the specific electrolyte

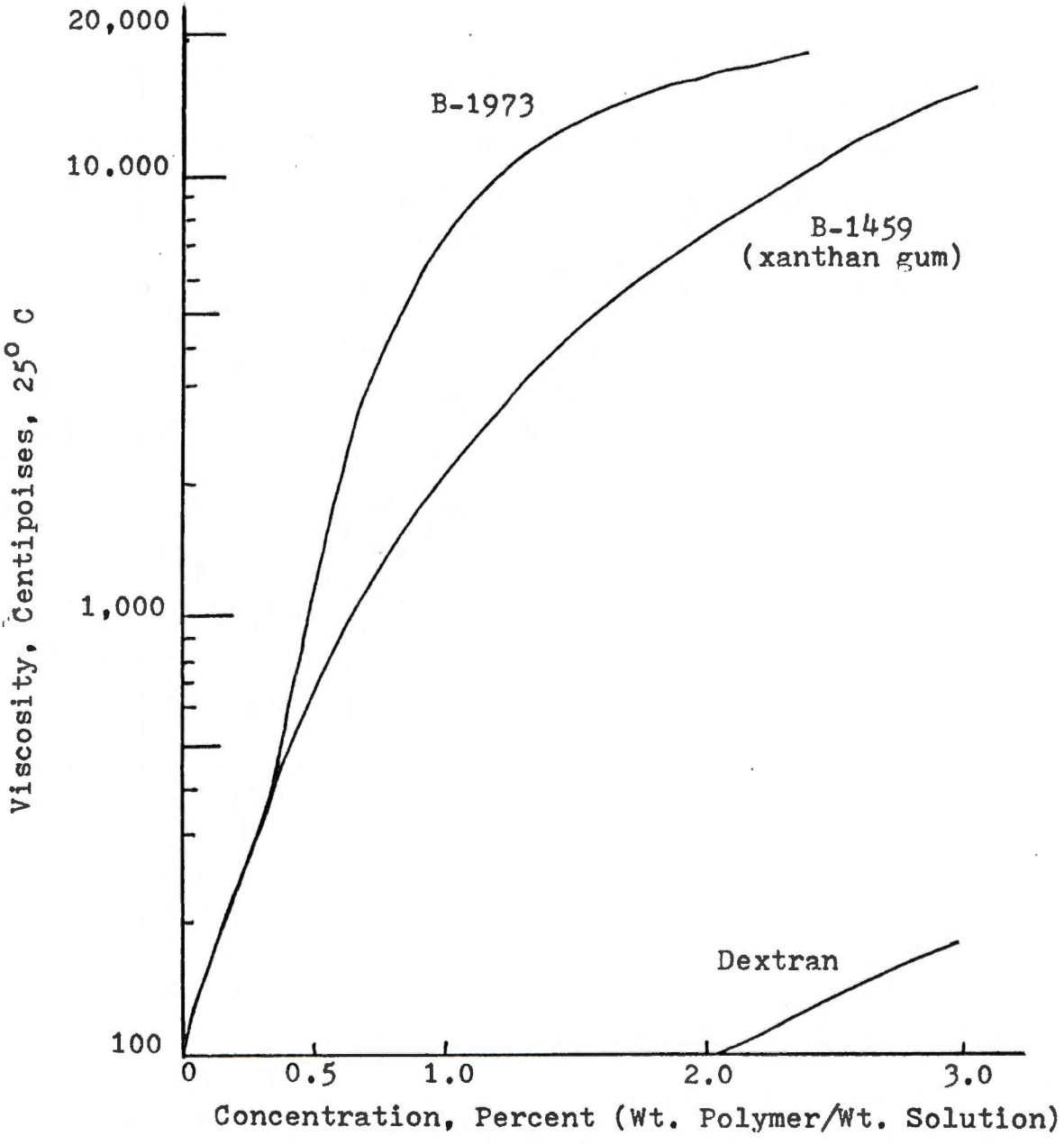

Figure 1. Viscosity concentration curves for microbial gums (21).

involved, the concentrations of both polymer and electrolyte, the ratio of these components, and the pH (13).

A restrained decrease in the viscosity of xanthan gum (1% aqueous solution) is seen at salt concentrations less than 0.3% but an increase at higher salt concentrations (21).

One percent aqueous solutions of polysaccharide B-1973 (8) double in viscosity upon the addition of NaCl or $CaCl_2$ at low concentrations, but at higher levels of either salt, viscosity is not significantly changed. However, when aluminum sulfate is added to the polymer solution at extremely low concentrations (.05%), viscosity is increased 20-fold and then decreases sharply as the salt level is increased.

Succinoglucan (17), an acidic polymer produced by Alcaligenes faecalis variety myxogenes, varies in its behavior to salts depending on the form of the polymer. The viscosity of the sodium (Na)-form is very sensitive to inorganic salts while that of the calcium (Ca)-form is very insensitive to them.

Studies have also investigated the effect of pH on viscosities of aqueous solutions of microbial gums. The viscosity of aqueous solutions of xanthan gum is nearly independent of pH between pH 6 and 9 and shows only minor variations over a pH range of 1 to 11 (27). Likewise, Jeanes et al. (23) found the viscosity of polysaccharide

B-1973 to be essentially constant in the range of pH 4 to 11. However, the effect of pH on succinoglucan is again dependent on the form of the polymer. The Ca-form is essentially independent of pH whereas the viscosity of the Na-form was practically zero at pH 5 through 9 and then rapidly increased below about pH 5 (17).

Stability of solution viscosity to heat is a property that has an important bearing on the applicability of any hydrophilic colloid.

Long exposures of xanthan gum to temperatures as high as 80° C appear to have little effect on viscosity, and resistance to degradation by heat can be improved by the presence of salts (13, 27). Solutions in the presence of KCl can be autoclaved at 121° C for 15 to 30 minutes with only minor viscosity changes (27).

However, Cadmus et al. (8) report that polysaccharide B-1973 has the ability to form a gel after autoclaving and cooling. Similarily, Harada et al. (18) isolated a beta-1,3-glucan from a mutant strain of Alcaligenes faecalis variety myxogenes which when heated in aqueous suspension to 100° C for a few minutes formed a firm resilient gel.

Both the Na- and Ca-forms of succinoglucan in the presence of NaCl showed increases in viscosity when held at 70° or 90° C for 10 minutes, although heat treatment at 70° C gave a higher effect (17).

Temperature stability of the viscosity of gum solutions is also in important property from the standpoint of food applications, and thus several studies have been conducted investigating this characteristic in solutions of microbial gums.

The solution viscosity of xanthan gum has been found to be nearly independent of temperature over a wide temperature range (27). Cadmus et al. (8) reported the viscosity of polysaccharide B-1973 solutions as increasing with temperature increases. However, Jeanes et al. (23) found decreases in viscosity of this polymer at high temperatures which were partially reversed upon spontaneous cooling. In the presence of KCl, viscosities remained constant throughout the temperature range. The Ca-form of succinoglucan as well as the Na-form in the presence of salt gave nearly zero solutions viscosities as the temperature approached 70° C (17).

III. IMPORTANCE OF MICROBIAL GUMS TO THE FOOD INDUSTRY

The interest that has been shown by the food industry in microorganisms as a new source of hydrocolloids stems from several considerations (22):

1. Successful performance of several of these microbial products based on their distinctive properties.
2. General suitability for ingestion without adverse effects because constituent natural residues and their pattern of glycosidic linkages permit either digestion and metabolism or inertness and non-caloric effect.

3. Economic feasibility resulting from extracellular occurrence of these biopolymers and their production in good yields from low cost substrates by fermentation procedures.
4. Need to supplement supplies of natural plant gums.
5. Various advantages of domestic products over foreign imports.

Numerous studies have demonstrated the applicability of microbial gums to food systems.

Dextran has found applicability in foods based on its ability to prevent crystallization, improve moisture retention, improve body, and maintain flavor and appearance in candies and jellies (25). It has also been noted that dextran has a high specific superiority for stabilizing texture in ice cream and sherbets (20) and has also been used as a stabilizer in various beverages and flavor extracts (26).

Bohn (5) found that incorporation of small quantities of dextran in yeast breads produced breads that were softer and had a greater volume and longer shelf life than ordinary breads.

Dextran has also been employed for preserving a large variety of foods by preventing the food from drying out during storage and by protecting it against deleterious effects of exposure to air (13).

Polysaccharide 13140 is a beta-1,3-glucan produced by a strain of Alcaligenes faecalis variety myxogenes. Kimura et al. (24) used this microbial gum successfully in noodles,

hamburgers, and sausage but suggested that it could be used as an additive or food material in any type of food, which, during production of cooking, is heated with water. Such foods include spaghetti, cereal flour foods, peanut butter, jellies, puddings, freeze-thaw foods, and dressings. Potential applications are based on its ability to improve viscoelasticity, palatability, binding quality, water-holding capacity, and compatibility with acid stability and heat and freeze-thaw stability (16).

In general, polysaccharide B-1973 displays unique viscosity characteristics that make it particularly advantageous as a thickening agent. More specifically, its responses to temperature and salts make it valuable for a diversity of food uses such as in specialty meats and pudding mixes (7).

The unusual properties of xanthan gum that have led to its various applications in foods are listed as follows (13):

1. Solubility in hot or cold water to give high viscosities at low concentrations.
2. Practically no change in viscosity with temperature variation.
3. Excellent solubility and stability in acid solutions.
4. Excellent stability in alkaline solutions.
5. Excellent suspending properties for hard-to-suspend solids.
6. Excellent compatibility and stability in the presence of salts.
7. Excellent heat stability even at high temperatures.
8. Extreme pseudoplastic properties.
9. Effective emulsifying properties.

Xanthan gum has been used effectively in citrus and fruit flavored beverages, canned food systems, frozen foods, relishes, and salad dressings (27). It has also been successful in dehydrated food products (10) and diet carbonated beverages (31) as well as in combination with other food gums such as locust bean for use in salad or dessert gels (33) and instant puddings (32).

Of equal importance to the food industry are the economics of microbial gums. With the advent of convenience foods, the demand for gums has been steadily increasing. And yet, shortages in plant gums due to crop failures and/or labor shortages have resulted in rising costs which have further been enhanced by inflation (28).

The food industry has already found two microbial gums, dextran and xanthan, to be economically feasible. It seems likely that as improvements in production and recovery of other microbial gums are found, they too will be commercially competitive.

IV. CHARACTERISTICS OF ALCALIGENES VISCOLACTIS

Alcaligenes viscolactis is a Gram negative short rod occurring singly, in pairs, or in short chains and is frequently found as an almost spherical cell. Capsules are produced in milk cultures, and in litmus milk, ropiness is produced, a pellicle is formed, and the reaction is alkaline.

Growth was reported to occur at 10° and at 20° C with variable growth at 37° and 40° C (6). However, Gainor and Wegemer (12) reported isolating a variant of A. viscolactis which had the ability to produce ropiness in milk at 5° C and the inability to do so at 36° C. In addition, the ease of capsule formation on simple nutrient agar at 5° C indicated the psychrophilic nature of the ropy isolate.

Although A. viscolactis was originally isolated from water and is still found in that environment, it also has a common habitat around dairy barns and dairy utensils.

Wegemer and Gainor (36) did further studies on the chemical nature of the capsular polysaccharide. Using an alcohol precipitation, they were able to isolate the polysaccharide. Hydrolysis of the material followed by thin layer chromatographic techniques showed the polysaccharide to be levan in nature.

V. IMPORTANCE OF WHEY UTILIZATION

The dairy foods industry has long been recognized as a significant contributor to stream pollution. About 10 years ago a United States Senate committee singled out the dairy industry as the second most important source of stream pollution, and whey has long been the most visible pollutant (11).

The potential significance of whey and the magnitude of the problem facing the industry is illustrated in the

statistics of whey production. Over 29 billion pounds of whey were produced in 1972, and the projections for 1980 are somewhere between 36 billion and 41 billion pounds (15).

Recognition of this problem resulted in the establishment of a whey utilization conference in 1970. Chairman Senti (34) in his opening remarks to the 1970 conference noted that :

> Heretofore economic considerations generally have not favored utilization of wastes (whey) as raw materials for product development. With the increasing concern about environmental quality, traditional methods of waste disposal can no longer be practiced and permissible disposal methods will entail considerably more expense.

An Environmental Protection Agency study (11) found that there is an increasing tendency for municipalities to set strength limits on waste water which are acceptable to being transferred to the treatment facility. The limits, frequently set at the level of municipal sewage, are not likely to be met by the dairy plants and may coerce the construction of separate treatment facilities.

The 1970 Whey Utilization Conference noted that complete recovery of whey in the United States would more than double the current supply of whey solids, thus necessitating a vigorous program of new products research and development (1). Likewise, the EPA study (11) recommended continued research in developing new methods for processing and marketing whey.

CHAPTER III

MATERIALS AND METHODS

I. SOURCE OF MATERIALS

One lot of dried cheddar cheese (sweet) whey was obtained from Kraftco Corporation to insure a uniform medium.

A strain of Alcaligenes viscolactis that was isolated from ropy milk was obtained from Dr. W. W. Overcast of the Food Technology and Science Department at the University of Tennessee, Knoxville.

II. PREPARATION OF WHEY

The dried sweet whey was analyzed for its moisture content, and the amount of water that was necessary to arrive at 6% total solids after rehydration was calculated. The dried whey was rehydrated accordingly.

The pH of the rehydrated whey was lowered to pH 4.7 with HCl, and the whey was steamed for 30 minutes to precipitate excess protein. The precipitated protein was removed by filtration through No. 12 Whatman filter paper.

The supernatant was neutralized to pH 6.8 with NaOH and dispensed into culture flasks in 200 ml portions. The flasks were brought up to 60° C in a water bath and then steamed for 10 minutes. Flasks were stored at 4° C until needed for inoculation.

III. ANALYSIS OF WHEY

Moisture

Moisture was determined by the vacuum drying method (2). Approximately 5 g of whey were dried in duplicate on a steam bath to remove visible moisture. The samples were then dried at 60° C for 20 hours at a vacuum of 381-508 torr to obtain a constant weight.

Ash

The dried samples were subjected to a heat of 550° C for 4 hours (2). After cooling to room temperature in a desiccator, samples were weighed, and the percentage of ash was calculated.

Protein

Protein was determined in duplicate by the Biuret method of Robinson (30). Two ml of whey were mixed with 2 ml of 28% Na_2SO_3 and 8 ml of Biuret reagent, and color was allowed to develop for 30 minutes. Absorbance was read on a Bausch and Lomb Spectronic 20 at 550 nm.

A standard curve had been constructed using Sodium Protolac (Borden Company), a dried whey product, for the standard. Protein as determined by Kjeldahl was 57.36% (9).

Fat

Fat was determined in duplicate by the Babcock method for skim milk as outlined by Goss (14).

Carbohydrate

Carbohydrate in the form of lactose was estimated by difference.

Statistical Analysis

Three replicates for each analysis were made. Data were subjected to analysis of variance (35).

IV. GROWTH OF ALCALIGENES VISCOLACTIS IN WHEY

Forty-eight hours prior to inoculation of the whey, 10 ml tubes of trypticase-soy broth were inoculated from the stock culture of Alcaligenes viscolactis and incubated at 21° C. The whey was then inoculated with 5% of these actively growing cultures and incubated at 21° C for 120 hours.

V. RECOVERY OF THE GUM

The culture fluid was diluted 1:4 with water, and 0.5 volumes of methanol was added. Dissolution of the gum was facilitated with the use of a magnetic stirrer.

The pH of the diluted culture fluid was lowered to pH 4.8-5.2 with HCl to precipitate the gum. The bulk of the precipitated gum was removed from the surface of the supernatant with a strainer, and the remainder was collected by filtration.

The gum was washed three times with a mixture of 60% acetone in water and then washed twice with acetone. The gum, suspended in acetone, was neutralized with NaOH. Excess acetone was decanted, and the gum was dried under flowing air.

Material from several runs was combined for a homogeneous experimental lot.

VI. ANALYSIS OF THE GUM

Approximately 1.5 g of the gum was weighed into a predried and preweighed thimble made from No. 3 Whatman filter paper. To determine moisture, the vacuum drying method (2) as described previously was used. The dried sample was then analyzed for fat by continuous extraction with petroleum ether and ethyl ether mixed in equal volumes using the Goldfisch apparatus (19). The defatted sample was analyzed for protein by the Biuret method (30) using 0.1 g of the sample dissolved in 100 ml of water. Sodium Protolac was again used as the standard. The remainder of the defatted sample was analyzed for ash (2). Carbohydrate was estimated by difference.

For plate counts, 0.1 g of the gum was suspended in 9.9 ml sterile water in a sterile bottle and dilutions were made as follows: 1:100; 1:1000; 1:10,000; 1:100,000; and 1:1,000,000. The dilutions were plated using Standard

Methods agar and incubated at 21° C for 48 hours.

All analyses were done in duplicate. For plate counts a second replication was made.

VII. RHEOLOGICAL PROPERTIES OF THE GUM

The effects of gum concentration, salt (NaCl) concentration, pH, heating treatment, and temperature of measurement on the viscosity of an aqueous solution of the gum were studied. The levels of each of these factors were as follows:

1. Gum concentration: 0.7, 1.4, 2.1% (w/v).
2. NaCl concentration: 0.5, 1.5, 2.5% (w/v).
3. pH: 5.8, 6.5, 7.2.
4. Heating treatment: 25°, 60°, 95° C for 10 min.
5. Temperature of measurement: 25°, 55°, 85° C.

The experiment was constructed as a 3^5 factorial in randomized blocks of 9 combinations for a total of 81 experimental units, representing 1/3 replication (4).

Aqueous solutions of the gum at the appropriate concentration were made up the day prior to testing to let the gum rehydrate sufficiently. Penicillin was added (1.25 µg per ml) to inhibit microbial growth during this rehydration period. On testing day NaCl was added in the appropriate amount, and the pH was adjusted accordingly. Each solution was heated to one of the above heating temperatures in a water or oil bath and held for 10 minutes (hereinafter

referred to as "heat"). The solution was cooled to 25° C by circulation of tap water and maintained at that temperature for 15 minutes. It was then equilibrated to one of the above temperatures by a circulating water bath providing the specified temperature for viscosity measurement (hereinafter referred to as "temperature"). All measurements were made with a Brookfield viscometer Model LVF using the small sample adapter equipment, spindle no. 18/34, and a speed of 30 rpm. Heating regimens were applied to samples in the small sample adapter holder.

Statistical Analysis

Accuracy and precision of the viscosity measurements were established by calculation of the standard deviation from five replicate measurements of a Brookfield viscosity standard (100 cps at 25° C).

Analytical error was assessed by randomly choosing nine combinations and repeating them. All randomizations were performed using a random number table.

Data were subjected to analysis of variance to measure treatment effects, block effects, two-factor interactions, and higher interactions. The data was used to construct a polynomial of the response surface for the interactions among the factors that were studied (29).

CHAPTER IV

RESULTS AND DISCUSSION

I. COMPOSITION OF WHEY

The composition of the whey that was used as the growth medium for Alcaligenes viscolactis is shown in Table I along with the standard deviation for each component.

Moisture was found to be 94.6% indicating a total solids content of 5.4%. Although the whey was originally rehydrated to 6% total solids, it should be noted that excess protein was heat-precipitated from the whey to prevent contamination of the gum. Avigad (3) has noted that in cases where the growth medium is rich in protein, the microbial gum may adhere to the protein and must be deproteinized. Thus protein was found to be 0.066% as compared to 0.8% normally found in sweet rennet whey (37).

The amount of fat in the whey was negligible being less than 0.01%. However, literature values for sweet rennet whey show the amount of fat to be 0.6% (37). Thus it might be assumed that most of the fat had been removed in combination with the excess protein.

Ash was found to be 0.510% and carbohydrate in the form of lactose was estimated by difference to be 4.824%. Both of these values are in agreement with those for sweet rennet whey (37).

TABLE I

COMPOSITION OF WHEY USED AS GROWTH MEDIUM FOR ALCALIGENES VISCOLACTIS

Component	Percent[a]	Std. Dev.
Moisture	94.600	±.060
Carbohydrate	4.824	±.178
Ash	0.510	±.100
Protein	0.066	±.018
Fat	<.010	--

[a]Average of six determinations

No differences at the 5% level of significance were found between replicates as determined by analysis of variance.

II. RECOVERY OF THE GUM

The gum that was produced by Alcaligenes viscolactis in the whey was generally found as a pellicle on the surface of the whey at the end of the incubation period. Dissolution of the gum into the whey after dilution with water could be brought about by mechanical treatment--either by shaking or magnetically stirring. The addition of acid to a pH 4.8-5.2 resulted in precipitation of the gum which after washing and drying exhibited the characteristic ropiness in aqueous solution.

Preliminary attempts had been made to use other more widely accepted methods for recovery of the gum. Alcohol precipitation, a common method used by several researchers (8, 17, 23, 36), was used as well as a specific method for water-insoluble microbial gums (18). Although in each case a fluffy white precipitate was obtained, this material no longer gave the characteristic ropiness when rehydrated, and, in fact showed no viscosity increase to the aqueous solution at all.

Yield of the dried gum was approximately 0.625 g per liter of culture fluid. In comparison with other microbial gums that have been isolated (8, 16), this is a rather low

yield. However, Jeanes (21) describes a microbial capsule, such as this, as a highly hydrated material, and thus a low dried weight might be accounted for in reference to this. It should also be noted that no attempts were made in this study to optimize growth conditions of the organism or techniques for recovery of the gum to produce an optimum yield. Thus it appears that further work in these areas is necessitated.

III. ANALYSIS OF THE GUM

The composition of the gum that was produced by Alcaligenes viscolactis is shown in Table II. The gum was found to contain 54.33% carbohydrate, 23.24% protein, 16.75% ash, 5.34% moisture, and 0.34% fat. The values for protein and fat may be explained in that no removal of the cells from the culture fluid prior to recovery of the gum was attempted. Preliminary studies showed that passing the diluted culture fluid through a Sharples centrifuge to remove the cells resulted in loss of the ropy characteristic. It has also been pointed out that there may be difficulty in isolating cell-free capsular materials such as that produced by A. viscolactis (21). Thus the protein and fat are probably cell constituents. However, some of the protein could possibly be a component of the gum. Jeanes (21) further describes a microbial capsule as a gelatinous material, indicating a proteinaceous nature.

TABLE II

COMPOSITION OF THE GUM PRODUCED BY *ALCALIGENES VISCOLACTIS*

Component	Percent[a]
Carbohydrate	54.33
Protein	23.24
Ash	16.75
Moisture	5.34
Fat	0.34

[a]Average of two determinations

Plate counts of the gum were made to determine the presence of viable A. viscolactis cells although it was thought that all cells would have been destroyed by the methanol and acetone in the recovery process. However, there were countable plates at the 1:1,000,000 dilution indicating high numbers of microorganisms. Gram stains of an isolated colony and of the A. viscolactis stock culture showed that a Gram positive long rod was present in the gum rather than viable A. viscolactis.

To prevent growth of the contaminant in the aqueous solutions of the gum to be prepared for viscosity measurements, the addition of an antibiotic to the solutions was considered. The contaminant was grown out in trypticase-soy broth and plated in Standard Methods agar with various levels of either Aureomycin or penicillin antibiotics. After an incubation period of 24 hours at 32° C, growth had been completely inhibited with penicillin in the amount of 1.25 µg per ml. Therefore, this level of penicillin was used accordingly in the aqueous solutions of the gum.

IV. RHEOLOGICAL PROPERTIES OF THE GUM

The effects of gum concentration, salt concentration, pH, heat, and temperature on the viscosity of aqueous solutions of the gum are shown in Table III as determined by analysis of variance. Linear and quadratic main effects as well as linear and quadratic two-factor interactions are

TABLE III

ANALYSIS OF VARIANCE OF EFFECTS OF GUM CONCENTRATION, SALT CONCENTRATION, pH, HEAT, AND TEMPERATURE ON VISCOSITY

Source	DF	SS	MS	F
Concentration	2	1407.30	703.65	243.52**
Conc., linear	1	1377.14	1377.14	476.60**
Conc., quad.	1	30.16	30.16	10.43**
Salt	2	20.38	10.19	3.52*
Salt, linear	1	9.80	9.80	3.39
Salt, quad.	1	10.58	10.58	3.66
pH	2	0.73	0.36	0.12
pH, linear	1	0.01	0.01	0.002
pH, quad.	1	0.72	0.72	0.25
Heat	2	116.81	58.40	20.21**
Heat, linear	1	58.49	58.49	20.24**
Heat, quad.	1	58.32	58.32	20.18**
Temperature	2	726.25	363.12	125.67**
Temp., linear	1	686.94	686.94	237.74**
Temp., quad.	1	39.31	39.31	13.60**
Conc. X Salt	4	18.21	4.55	1.58
C X S	1	6.76	6.76	2.34
C X S2	1	7.36	7.36	2.55
C2 X S	1	2.31	2.31	0.80
C2 X S2	1	1.78	1.78	0.62
Conc. X pH	4	5.32	1.33	0.46
C X p	1	2.35	2.35	0.81
C X p2	1	2.43	2.43	0.84
C2 X p	1	0.21	0.21	0.07
C2 X p2	1	0.32	0.32	0.11
Conc. X Heat	4	42.40	10.60	3.67*
C X H	1	4.07	4.07	1.41
C X H2	1	29.14	29.14	10.08**
C2 X H	1	0.09	0.09	0.03
C2 X H2	1	9.10	9.10	3.15
Conc. X Temp.	4	230.13	57.32	19.91**
C X T	1	181.80	181.80	62.92**
C X T2	1	35.71	35.71	12.36**
C2 X T	1	3.52	3.52	1.22
C2 X T2	1	9.10	9.10	3.15
Salt X pH	4	13.26	3.31	1.15
S X p	1	2.61	2.61	0.90
S X p2	1	5.83	5.83	2.02
S2 X p	1	4.69	4.69	1.62
S2 X p2	1	0.12	0.12	0.04

TABLE III (continued)

Source	DF	SS	MS	F
Salt X Heat	4	10.81	2.70	0.94
S X H	1	3.61	3.61	1.25
S X H2	1	2.20	2.20	0.76
S2 X H	1	1.00	1.00	0.35
S2 X H2	1	4.00	4.00	1.38
Salt X Temp.	4	8.79	2.20	0.76
S X T	1	1.32	1.32	0.46
S X T2	1	1.74	1.74	0.60
S2 X T	1	5.47	5.47	1.89
S2 X T2	1	0.27	0.27	0.09
pH X Heat	4	8.93	2.23	0.77
p X H	1	0.61	0.61	0.21
p X H2	1	6.90	6.90	2.39
p2 X H	1	1.36	1.36	0.47
p2 X H2	1	0.06	0.06	0.02
pH X Temp.	4	9.76	2.44	0.84
p X T	1	5.68	5.68	1.96
p X T2	1	0.30	0.30	0.10
p2 X T	1	0.10	0.10	0.03
p2 X T2	1	3.67	3.67	1.27
Heat X Temp.	4	30.48	7.62	2.64
H X T	1	0.47	0.47	0.16
H X T2	1	0.16	0.16	0.05
H2 X T	1	24.94	24.94	8.63**
H2 X T2	1	4.91	4.91	1.70
Residual	30	86.68	2.89	
Total	80	2736.22	34.20	

Y = linear effect
Y2 = quadratic effect
** = significant at 1% level
* = significant at 5% level

presented. Block effect was found to be insignificant based on the nine replications that were made and, thus, was combined with the residual error term.

Concentration of the gum gave a highly significant positive effect with viscosity increasing as the gum concentration increased. Most of this variation was found to be linear in nature; however, quadratic variation was also found to be significant. The viscosity-concentration relationship is typical of several microbial gums (8, 13, 16, 17, 21).

Salt concentration had a significant negative effect on the viscosity of the aqueous gum solution. However, when the sum of squares for this effect was partitioned to show the type of variation, neither the linear or quadratic effects were significant. The effect of salt on the viscosity of other microbial gum solutions shows much variation depending on the particular salt used, the ratio of gum and salt concentration, and even the chemical form of the gum (8, 13, 17, 23).

The effect of pH was not significant in altering viscosity at the levels that were used. This lack of effect is a characteristic that generally has been found with microbial gums (8, 13, 17, 21, 23). However, the use of a broader pH range might have resulted in greater variation.

Heat was found to have a highly significant negative effect causing viscosity to decrease. This variation was

equally divided between linear and quadratic as can be seen by the partitioned sum of squares (Table III).

The effect of temperature was also negative and highly significant. The greater portion of this variation was a linear effect; however, quadratic variation was also significant. This characteristic seems to be typical of the Alcaligenes microbial gums; succinoglucan also shows a decrease in viscosity with an increase in temperature (17).

In the two-factor interactions only concentration by heat and concentration by temperature showed significant effects. In the interaction of concentration and heat, the effect was largely due to the linear concentration by quadratic heat portion of the partitioned sum of squares. In the concentration-temperature interaction, significant effects were largest in the linear interaction of the two factors, but effects were also significant for linear concentration by quadratic temperature.

The interaction of heat with temperature was in general not significant. However, in partitioning the sum of squares, the interaction between a quadratic variation in heat and a linear variation in temperature was significant.

From the magnitude of the F values in Table III, concentration had the greatest effect on viscosity out of all the factors and interactions that were studied. The other main factors could be ranked as follows: temperature

> heat > salt > pH. Salt was just barely significant at the 5% level, and pH was not a significant effect.

Likewise, the interaction of concentration by temperature had a greater effect on viscosity than did concentration by heat; the former was significant at the 1% level and the latter, at the 5% level.

The viscosities that were obtained ranged from 1.0 to 32.4 centipoise. In comparison with the viscosities of aqueous solutions of other microbial gums (8, 13, 16, 17 21, 27), these values were low. However, since concentration had the greatest effect on viscosity and this effect was in the positive direction, it seems likely that much higher viscosities would have been obtained if the tested concentrations had been somewhat higher. This gum, then, would be similar to dextran gum which requires a 15% concentration to be comparable in flow behavior to that of 1% solutions of some other microbial gums (22).

Based on the results obtained from the analysis of variance table, a model was designed for deriving a regression polynomial. Twelve of the factors and interactions that were found to be significant were used in order to establish a simplified model containing only significant effects. Since the effect of salt overall was found to be significant, the linear and quadratic effects of salt were included in the model although these were not significant

effects individually. The derived polynomial was used to compute predicted viscosity values.

The plot of predicted viscosities versus observed viscosities is shown in Figure 2 along with the correlation coefficient, regression polynomial, and standard deviation. In general, there was a high correlation between observed and predicted viscosities with a correlation coefficient of 0.91. Had the model been designed to contain both significant and nonsignificant effects, a correlation coefficient of 0.96 would have been obtained. Thus only a slight degree of certainty was sacrificed in using the simplified model.

It is evident from Figure 2 that two data points, (26.8, 21.3) and (32.4, 22.2), did not fit the model. Whether these are the result of some unknown experimental error or possibly some higher order interactions cannot be determined at this time. However, since the majority of the data points follow a strict linear arrangement, higher order interactions seem unlikely. Further experimental work is warranted if these discrepancies are to be resolved.

The regression analysis generated with the 12 factor model showed an error (residual) sum of squares of 239.63. The two data showing the wide variation accounted for 56% of the variation from regression, more than half the residual sum of squares.

The standard deviation obtained from the analysis was ± 1.88. However, calculation of the standard deviation

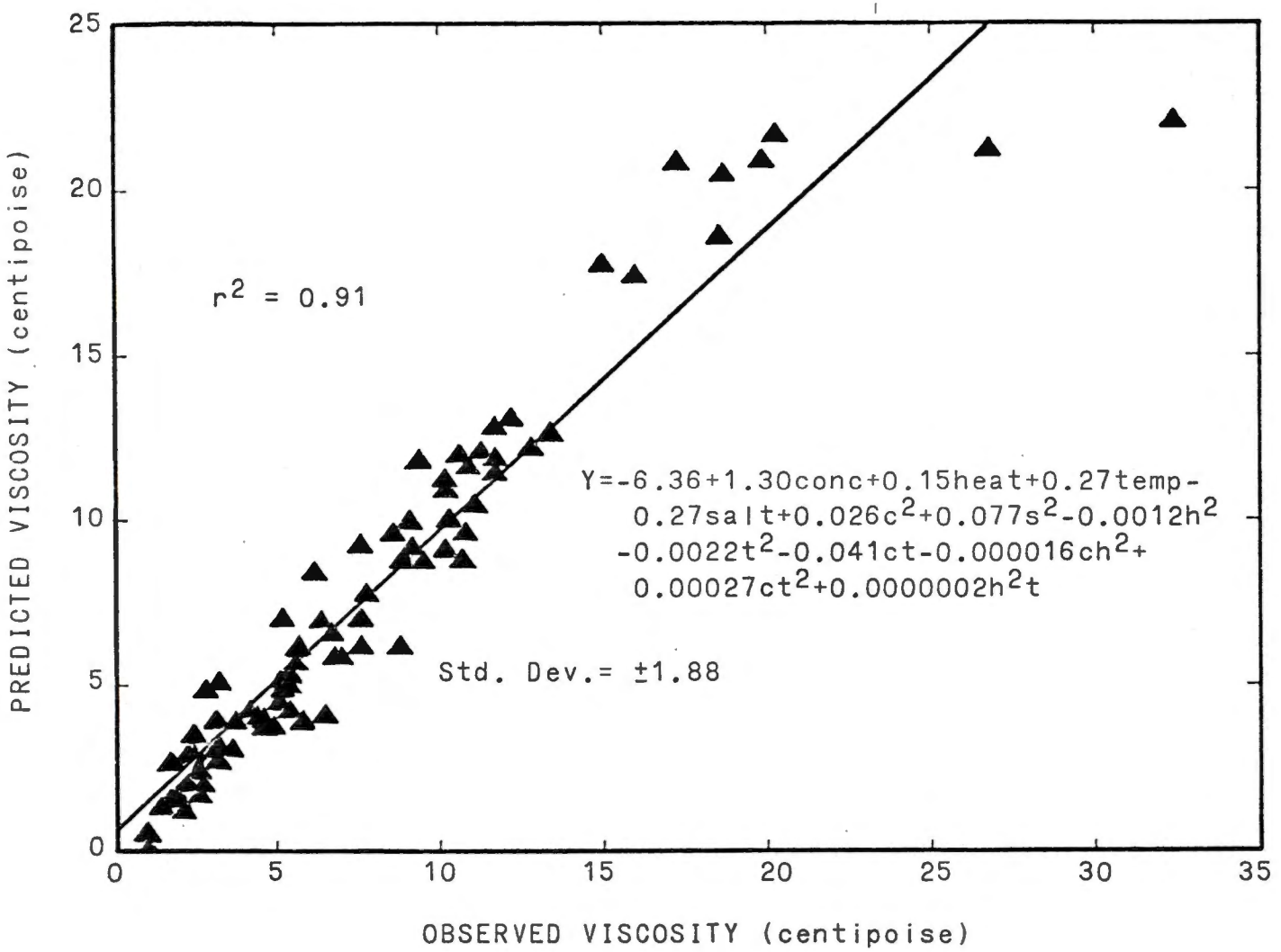

Figure 2. Plot of predicted viscosities versus observed viscosities.

based on the replication of nine randomly chosen treatments was found to be ±0.33 (Table IV). This value was well within the limits established by the five replicate measurements made with the viscosity standard for which the standard deviation was ±0.62 (Table V). Thus, these two variant data points might be instrumental in explaining this difference.

The feasibility of using the gum produced by A. viscolactis for food applications does not appear to be substantiated by the results of this study. The low yield that was obtained would seem to discourage any economic considerations although this aspect might be made more favorable with further study into the growth of the organism and recovery of the gum. The rheological properties of the gum also failed to show any outstanding characteristics. The gum was somewhat difficult to get into solution and did not produce any dramatic increases in viscosity as has been witnessed with some other microbial gums. However, it should be noted that dextran has this same low viscosity characteristic and yet has found wide application. Thus, possibly with further study into other functional properties such as emulsifying capacity and stability and the ability to form films, the gum produced by A. viscolactis may find its niche in the food industry.

TABLE IV

VISCOSITIES OF NINE TREATMENTS RANDOMLY CHOSEN FOR REPLICATION

Treatment Number	Viscosity (cps)[a] Rep. 1	Rep. 2
3	7.8	7.8
8	3.2	2.8
19	3.4	3.7
23	7.4	7.0
28	11.8	12.2
34	5.4	5.4
35	4.6	4.4
41	8.4	8.9
47	2.4	2.6

[a]spindle 18/34, speed 30 rpm

Std. Dev. = $\pm$0.33

TABLE V

PRECISION OF BROOKFIELD VISCOMETER WITH VISCOSITY STANDARD

Sample	Viscosity (cps)[a]
1	100.00
2	100.00
3	99.75
4	98.50
5	99.50

[a] 25° C, spindle 18/34, speed 12 rpm

Std. Dev. = ± 0.62

CHAPTER V

SUMMARY

The purpose of this investigation was to isolate the gum produced by the non-pathogenic microorganism Alcaligenes viscolactis when grown in whey and to characterize the rheological properties of the gum as they pertain to food systems.

The whey that was used as the growth medium for the organism was prepared by rehydrating dried sweet whey and removing excess protein by heat precipitation. Thus, the composition of the rehydrated whey was found to be 94.600% moisture, 4.824% carbohydrate, 0.510% ash, 0.066% protein, and less than 0.010% fat.

A 5% inoculum of A. viscolactis was grown in the whey for 120 hours at 21° C, after which time the gum was formed as a pellicle on the surface of the whey. After complete dissolution into the culture medium, the gum was recovered by acid precipitation at a pH of 4.8-5.2. The gum was washed with water and acetone and dried by flowing air. The composition of the gum was found to be 54.33% carbohydrate, 23.24% protein, 16.75% ash, 5.34% moisture, and 0.34% fat.

Rheological properties of the gum were investigated by studying the effects of gum concentration, salt (NaCl)

concentration, pH, heating treatment, and temperature of measurement on the viscosity of aqueous solutions of the gum. A 3^5 factorial in randomized blocks of 9 combinations for a total of 81 experimental units with 1/3 replication was used as the experimental design.

Twelve factors and interactions were found to have a significant effect on viscosity. These were the linear and quadratic effects of concentration, salt, heat, and temperature and the interactions of concentration by temperature; concentration by heat, quadratic; concentration by temperature, quadratic; and heat, quadratic by temperature.

Using these 12 factors and interactions, a regression polynomial was derived. Correlation between predicted and observed viscosities was high with a correlation coefficient of 0.91.

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VITA

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