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I am submitting herewith a thesis written by Sung-Hsiung Tsai entitled "A Whey Substrate as a Source for Xanthan Gum." 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.

W. W. Overcast, Major Professor

We have read this thesis and recommend its acceptance:

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A WHEY SUBSTRATE AS A SOURCE FOR XANTHAN GUM

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Sung-Hsiung Tsai

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ABSTRACT

Xanthomonas campestris grew abundantly in neutralized acid whey indicating the acid whey contained the necessary nutritional requirements of this microorganism.

Polysaccharide production by Xanthomonas campestris in one control and twelve acid whey media was evaluated. There was no significant difference among replications but there was a significant difference at 1 percent level among the thirteen media in the effect on the polysaccharide production. Medium 1 was significantly different from all others and superior at 5 percent level. In case of acid whey media, medium 13 was significantly different from all other acid whey media except medium 12. This indicated that medium 13 was superior to the other acid whey media for production of polysaccharide except medium 12.

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CHAPTER I

INTRODUCTION

Whey is a by-product of the cheese industry. About one-half of the whey is used, mostly in feed and food, and one-half is waste. Acid whey from the manufacture of many varieties of cheese is a product difficult for disposal. Disposal of such whey has received considerable attention to develop suitable uses or economical disposal.

Whey contains several valuable nutrients usable for growth of microorganisms. The whey protein contains all of the essential amino acids. Lactose contained in whey may be used as a source of carbohydrate in fermentation. Whey also supplies the nitrogen, vitamins and other growth requirements of many microorganisms.

The polysaccharide hydrocolloids, used traditionally for many purposes in foods and food processes, have been obtained from plants of both land and sea. A new additional source of polysaccharides is based on the biosynthetic capability of microorganisms. Xanthan gum is one of these microbial polysaccharides. Many species of Xanthomonas produce viscous gummy colonies. More than 100 species have been reported, but the species Xanthomonas campestris is reported to be the best producer of extracellular polysaccharide (17). Xanthan gum, polysaccharide B-1459, is a polymer produced by Xanthomonas campestris in a fermentation medium of 3 percent dextrose, 0.4 percent distillers' dried solubles, 0.5 percent dipotassium hydrogen phosphate, 0.01 percent magnesium sulfate heptahydrate. The nutritional requirements for polymer production are

furnished by distillers' dried solubles. The possible use of acid whey as a fermentation medium for production of polysaccharide gum would appear feasible.

Therefore this study describes the production of xanthan gum with the objectives to determine the feasibility of acid whey as a medium for xanthan gum production and to compare the acid whey media with distillers' dried solubles medium.

CHAPTER II

LITERATURE REVIEW

I. QUANTITIES OF WHEY PRODUCED

The largest pollutant in dairy food plant waste waters is whey. Whey is the serum or watery part of milk that separates from the curds in the process of making cheese. It is the largest by-product of the huge United States dairy industry, and is one of the most troublesome by-products produced by any industry.

Whole milk is used to produce natural and processed cheese such as Cheddar and the resultant fluid is called sweet whey with a pH in the range of 5 to 7. Skim milk is used to produce Cottage cheese and the fluid by-product is called acid whey with a pH in the 4 to 5 range (18). The low pH results from acid developed for coagulation. Each pound of cheese produced results in 5-10 pounds of fluid whey. About 70 percent of the nutrients in skim milk is part of the acid whey (6, 19).

Approximately 32 billion gallons of wash water, 3.5 billion gallons of cheese whey and 66 billion gallons of condenser water are discharged from the nation's dairy plants each year. Only about one-third of the whey produced each year is used to any extent. The remainder is discharged from the plants. The nutritive content of whey offers interesting possibilities for by-product recovery and utilization if suitable equipment and markets are available (6, 11, 19, 39).

II. COMPOSITION AND NUTRITIVE VALUE OF WHEY

The composition of typical cheese whey contains 7 percent total solids, of which 0.6 percent is ash and 6.4 percent is organic solids. The organic solids are divided among the fat at 0.3 percent, nitrogenous compounds 0.9 percent, lactose 4.9 percent and lactic acid 0.2 percent (25). About 56 percent of the nitrogenous matter is heat-coagulable protein. This protein commonly is called whey protein. The whey proteins have excellent nutritional quality with all of the essential amino acids (36, 38, 40). The ash constituents present in whey are: 0.118 percent potassium oxide, 0.075 percent sodium oxide, 0.071 percent calcium oxide, 0.018 percent magnesium oxide, 0.001 percent ferric oxide, 0.110 percent phosphorus pentoxide, 0.107 percent chloride and 0.029 percent sulfur trioxide (38). Of these, the calcium and phosphorus have valuable nutritional qualities (38). The nitrogenous compounds consist of amino acids, ammonia, creatine, creatinine, lactoglobulin, other proteins, peptones, urea and uric acid. Lactose is used as a source of carbohydrate in many industrial fermentations and possibly whey could be used as a substitute. In whey the nutrients that are present in addition to lactose are sufficient to supply the nitrogen, vitamins and other growth requirements of microorganisms (27, 38).

III. UTILIZATION OF WHEY AS A CULTURE MEDIUM

Because whey contains various nutrients and lactose, whey is employed as a culture medium and a substrate for fermentation (5, 21). In 1896 Storch (34) indicated that whey was suitable for the propagation of

organisms responsible for aroma formation in butter. Chernomordik and Gurina (3) reported that whey could be substituted for lactose in some bacteriological media. Wright and coworkers (42) found that some strains of Lactobacillus bulgaricus did not grow in a medium containing vitamin B_{12} and yeast extract unless dried whey was added. Whey has been employed as a substrate for the propagation of Penicillium roqueforti. Lundstedt (20) noted that cottage cheese whey treated with citrate stimulated the growth of heterofermentative lactic streptococci (Leuconostoc). Similar reports have also shown the use of whey for a growth media (4, 42). Whey was also suitable for growth of Saccharomyces fragilis. Yeasts, including Torula cremoris, Candida tropicalis, Candida utilis, Torula lactosa and Torulopsis sphaerica, were satisfactory as fermenters of lactose in whey (10).

Whey has been suggested as a substrate for use in the manufacture of yeast, alcohols, lactic acid, vitamins, acidic and alcoholic beverages, vinegar and other fermentation products (27, 41).

IV. HYDROCOLLOIDS

Over the past 50 years, many hydrocolloids have been widely used in the food industry (Table I). Polysaccharides, which currently are used industrially, are obtained chiefly from plant sources of both land and sea. These materials include cellulose, starch, pectins and other plant gums (7, 8).

A new additional source of polysaccharides is based on the biosynthetic capabilities of microorganisms (13). Xanthan gum is one

TABLE I

HYDROPHILIC COLLOIDS OF IMPORTANCE
TO THE FOOD INDUSTRY

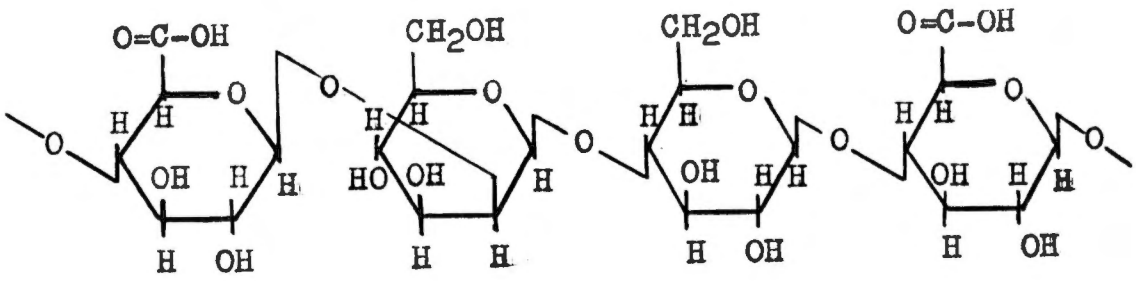
PLANT EXUDATES	OTHER
Gum tragacanth	Pectin-low methoxyl pectin
Gum arabic	
	SEAWEED EXTRACTS
PLANT SEED GUMS	
	Carrageenan
Locust bean gum	Furcellaran
Guar gum	Agar
	Algin-propylene glycol alginate
SYNTHETICS	
	FERMENTATION PRODUCTS
Sodium carboxymethylcellulose	
Hydroxypropyl cellulose	
Methylcellulose	Xanthan gum

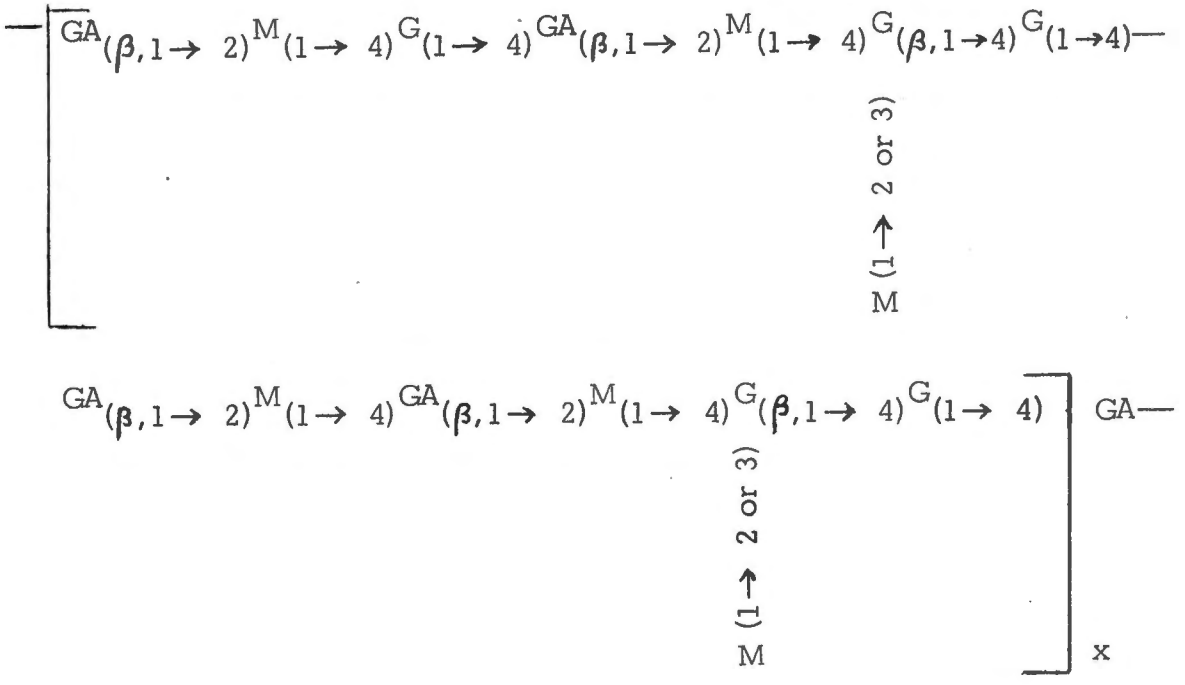
(Reference 28)

of these biosynthetic polysaccharides. Xanthan gum was successfully developed by the Northern Regional Laboratory at Peoria, Illinois (29). The polysaccharide B-1459, xanthan gum, has such interesting properties that several industrial companies investigated its commercial possibilities (28). The Kelco Company started the commercial production, while Archer Daniels Midland Company, Eli Lilly Company and Commercial Solvents Corporation have explored the commercial potential of this material for food, drug and cosmetic applications. Xanthan gum is produced by fermentation, using a pure culture of Xanthomonas campestris with dextrose as the substrate, followed by purification and recovery by precipitation with alcohol (12, 17, 29).

V. STRUCTURE OF XANTHAN GUM

Xanthan gum is a heteropolysaccharide with a molecular weight of more than one million. The polymer contains D-glucose, D-mannose and D-glucuronate in the molar ratio of 3:3:1. It also contains about 4.7 percent O-acetyl groups and about 3 percent pyruvic acid present as a ketal on the glucose units. Xanthan gum contains a basic repeating unit of 16 residues of which 13 are present in the main linear chain with 3 residues attached as single unit side chains. The D-glucuronic glycosidic linkages are β (1 → 2). D-glucose and D-mannose glucosidic linkage in the main linear chain are (1 → 4) and all appear to have the β-configuration. Mannose single unit side chains are attached to the main chain by (1 → 2)- or (1 → 3)-glycosidic bonds (31, 32). The structure of xanthan gum is given in Figure 1.





GA = glucuronic acid (as glucuronate)
M = D-mannose
G = D-glucose

Also present: 3.4 moles o-acetyl, 5.0 moles K^+ and Na^+ (As salts of glucuronic and pyruvic acids), and 1.0 mole pyruvate-substituted D-glucose.

Figure 1. Structure of xanthan gum (Sloneker and coworkers 31)

VI. PROPERTIES OF XANTHAN GUM

High Viscosity at Low Concentration

Xanthan gum dissolves readily in hot or cold water to give a high viscosity solution at low concentrations. The viscosity of a 1 percent solution is about 800-1000 centipoises when measured at 60 RPM with a Brookfield Model LVF viscometer at 25°C (12).

Solutions Are Pseudoplastic

At rest, viscosity is high, but as shear rate or agitation increases viscosity decreases. This is an instantaneous and reversible process. At 60 RPM the viscosity of a 1 percent solution is around 1000 centipoises. At 12 RPM the viscosity is more than 3000 centipoises and at 6 RMP is in the 5000 centipoises range (12).

Solutions containing 0.75 percent or more of xanthan gum have a rheological yield point. That is, the solution behaves as a solid until a minimum shearing force is reached. At shearing stresses above this minimum, the system behaves as a liquid (23, 28). Aqueous solutions of xanthan gum lose viscosity with increasing shear rate; the gum appears to have a better mouthfeel and is not as slimy as most other gums (35). Because of its pseudoplastic nature, xanthan gum is an excellent suspending agent even at very low levels.

Good Temperature Stability

Temperature has very little effect on the viscosity of xanthan gum solutions. They can be held at elevated temperatures for long periods of time without any major change in viscosity. In addition, the gum

solution is very stable to heat in the presence of potassium chloride and other salts (9, 12).

Xanthan gum solutions are also quite stable to freezing conditions. At very low levels, on the order of 0.1 to 0.2 percent, xanthan gum gives outstanding freeze-thaw stability to many food products such as puddings and spoonable-type dressings (28).

Independent of pH Effect

The viscosity of xanthan gum solutions in water is essentially independent of pH and the dry polymer can be dissolved directly in most acid solutions. Because of this nature, xanthan gum is of value in many food products. In the food industry, xanthan gum is being used as a thickener in foods with a range of pH values (28).

Good Compatibility with Salts

As mentioned above, salts improve the heat stability of xanthan gum solution. They also have a minor effect on viscosity. Solutions containing over 0.5 percent xanthan gum will increase in viscosity upon the addition of a trace of salt (12). Xanthan gum has good compatibility with high concentrations of many salts and dissolves directly in solutions that contain a moderate concentration of salt. By adding the salt or salt solution to a predissolved xanthan gum, it will then be compatible with up to 25 percent solutions of aluminum sulfate, calcium chloride or zinc chloride (23, 24, 28). Incompatibilities are few, but problems may occur under certain conditions; for example, calcium salts at above pH 10 precipitate xanthan gum (22). Long-chain quaternary ammonium salts

or amines with more than eight carbon atoms in the main chain may precipitate xanthan gum (30).

Xanthan gum will dissolve in 5 percent solutions of acetic acid, nitric acid and sulfuric acid, in 10 percent solutions of hydrochloric acid and in 25 percent solutions of phosphoric acid. Such solutions are quite stable for several months unless the temperature is elevated (23).

Film Formation

Xanthan gum, either native, heated or deacetylated, can form unsupported films of good quality. Deacetylated xanthan gum forms stronger and more flexible films than the native polysaccharide. These films were cast from a deaerated 1 percent aqueous solution in which glycerol was used as the plasticizer to reduce brittleness (37).

Effect on Oil-Water Emulsions

Small amounts of xanthan gum improve the stability of oil-in-water emulsions (26). Xanthan gum can be used as a stabilizer and has wide application for both pourable and spoonable salad dressings. This stability will exist even at elevated temperature because of the pseudoplastic nature of xanthan gum solutions (28).

Gel Reactions

Although xanthan gum does not interact with most other gums, it reacts with locust bean gum to form a gel (28). At low concentrations the increase in viscosity is highly significant and as the colloid concentration is increased, a thermoreversible and highly cohesive gel is obtained.

The higher the pH, the firmer the gel, but practical applications for these gels are also being found at pH as low as 3.5. At this pH, the gel has a somewhat softer body than at neutral pH. The texture of these gels can be modified by the addition of other gums and particularly by starch (15, 28).

VII. FOOD APPLICATIONS OF XANTHAN GUM

Beverages

Xanthan gum is an excellent suspending agent for the desirable cloud and pulp in some drinks. Xanthan gum also is promising as a stabilizer for the flavor oil emulsions employed in certain of these beverages. Concentrations of xanthan gum in the finished drink may range from 0.001 percent to 0.15 percent (2, 14).

Canned Foods

Because of its unique heat stability combined with excellent emulsion stabilizing and suspending properties, xanthan gum is quite useful in canned food systems (14).

Frozen Foods

Frozen starch-based pie fillings and puddings may show syneresis and retrogradation after one or two freeze-thaw cycles. The addition of 0.1 percent of xanthan gum gives a dramatic improvement in freeze-thaw stability (14).

Relishes

Pickle, tomato, onion and mustard relishes impose severe requirements on the stabilizer used. These systems may contain high salt, high acidity, or a combination of the two. Xanthan gum not only is compatible with these systems, but is an excellent stabilizer, suspending agent and thickening agent under these adverse conditions. Concentrations of 0.1 percent xanthan gum are effective in such relishes (14).

Salad Dressings

Xanthan gum, at concentrations considerably lower than other competitive gums, imparts superior emulsion stability, easier pumpability, less-gummy mouth feel, more cling, excellent pourability at refrigerated temperature, and better flavor release due to its pseudoplastic nature. The partial replacement of starch with xanthan gum affords a less-pasty mouth feel, resulting in better flavor release (14, 24).

Xanthan Gum—Locust Bean Gum Gels and Puddings

Xanthan gum in combination with locust bean gum provides easy gel formation for preparation of puddings and pie fillings. The partial starch replacement with xanthan gum and locust bean gum affords less-pasty mouth feel, better texture, and improved flavor release (14).

CHAPTER III

MATERIALS AND METHODS

I. MAINTENANCE OF PURE CULTURE

Xanthomonas campestris NRRL B-1459 was obtained from the American Type Culture Collection where it was maintained as a lyophilized stock culture. Viable cultures for inoculum build-up were maintained on malt extract—yeast extract agar slants and were transferred weekly to minimize lag in the inoculum. Freshly inoculated slants were incubated at 25°-28°C for 2 days, then refrigerated for routine use.

II. PREPARATION OF INOCULUM

In the preparation of inoculum, growth from a slant was transferred to malt extract—yeast extract broth which was adjusted to pH 7.0 with 1 N sodium hydroxide. The inoculum was incubated 24 hours on the reciprocating shaker at 25° -28°C and 5 percent of inoculum was used to seed the flasks of experimental media.

III. PREPARATION OF ACID WHEY

Skim milk was steamed for one hour then cooled to room temperature. Steamed skim milk was then seeded with 1 percent of H-44 lactic acid culture and incubated at 21°C for 16 hours. After incubation, the coagulated skim milk was stirred to break up the coagulum and steamed for 15

minutes. The curd was separated from the whey by filtering through double thickness cheese cloth. The greenish yellow filtrate was the acid whey.

IV. PREPARATION OF MEDIA FOR FERMENTATION

Thirteen prepared media were used for production of xanthan gum. These media were prepared as follows:

1. Control medium contained 3 percent dextrose; 0.4 percent distillers' dried solubles; 0.5 percent dipotassium hygrogen phosphate; and 0.01 percnet magnesium sulphate heptahydrate. pH was adjusted to 7.0 with 2 N hydrochloric acid.

2. Acid whey (all acid whey or whey media were adjusted to pH 7.0 with saturated sodium hydroxide).

3. Acid whey with 0.07 percent lactase (lactase, B-D-galactoside galactohydrolase, E. C. 3. 2. 1. 23., obtained from Enzyme Development Company, New York, New York, was added in the neutralized acid whey and incubated at 4°C for 16 hours).

4. Acid whey with 0.4 percent distillers' dried solubles.

5. Acid whey with 0.07 percent lactase; 0.4 percent distillers' dried solubles.

6. Acid whey with 0.5 percent dipotassium hydrogen phosphate; 0.01 percent magnesium sulphate heptahydrate.

7. Acid whey with 0.07 percent lactase; 0.5 percent dipotassium hydrogen phosphate; 0.01 percent magnesium sulphate heptahydrate.

8. Acid whey with 0.4 percent distillers' dried solubles; 0.5 percent dipotassium hydrogen phosphate; 0.1 percent magnesium sulphate heptahydrate.

9. Acid whey with 0.07 percent lactase; 0.4 percent distillers' dried solubles; 0.5 percent dipotassium hydrogen phosphate; 0.01 percent magnesium sulphate heptahydrate.

10. Acid whey with 3 percent dextrose.

11. Acid whey with 3 percent dextrose; 0.4 percent distillers' dried solubles.

12. Acid whey with 3 percent dextrose; 0.5 percent dipotassium hydrogen phosphate; 0.01 percent magnesium sulphate heptahydrate.

13. Acid whey with 3 percent dextrose; 0.4 percent distillers' dried solubles; 0.5 percent dipotassium hydrogen phosphate; 0.01 percent magnesium sulphate heptahydrate.

By this scheme the factors contained in the control medium were added sequentially and in all combinations to whey with the added variable of hydrolyzing part of the lactose.

V. FERMENTATION

Media for fermentation were autoclaved at 121°C for 15 minutes, then seeded with 5 percent inoculum of Xanthomonas campestris NRRL B-1459. Media were incubated on a reciprocating shaker at 26° -28°C for 96 hours (29) in 1000 ml. Erlenmeyer flasks containing 300 ml. of medium used for the experiment.

VI. MEASUREMENT OF APPARENT VISCOSITY

Apparent viscosity of the fermented broth was measured with a Brookfield Synchro-Lectric Viscometer LVF Model. The No. 1 LVF spindle was

used for all measurements. The spindle rotated at 6 RPM and the temperature of the fermented broth was at room temperature. The methods as outlined by the manufacturer (1) were followed to measure the viscosity of the fermented broth.

VII. RECOVERY OF POLYSACCHARIDE

The pH of the incubated media was adjusted to 5.6 with 2 N hydrochloric acid and 3 volumes of water and 24.7 percent methanol were added. Cells and suspended impurities from this media were removed by a continuous Sharples Supercentrifuge. A soft gel-like crude polymer was precipitated from the centrifugate by adding 2 percent potassium chloride and additional methanol to 50 percent. The precipitate was recovered by centrifuging at 3500 × g for 60 minutes. The crude polysaccharide was purified by filtering the aqueous solution containing 0.5 volume of methanol and lowering the pH to 4.7 with 2 N hydrochloric acid. The polymer was reprecipitated by adding 2 percent potassium chloride to the filtrate and centrifuging at 3000 × g for 30 minutes. The polymer was decolorized and dehydrated by suspending the gum in 100 ml of methanol and filtering. Residual methanol was removed by vacuum drying at 18.5 inches Hg and 40°C and the polymer was weighed (29).

VIII. STATISTICAL ANALYSIS

The experiment was a 13 × 3 (treatment by replication) factorial of a complete block design. Data were analyzed by analysis of variance (16). Significant difference among means was determined by use of Duncan's Multiple Range Test (33).

CHAPTER IV

RESULTS AND DISCUSSION

Xanthomonas campestris grew abundantly in the neutralized acid whey indicating the whey contained the necessary nutritional requirements of this microorganism. Polysaccharide production in twelve different acid whey media was evaluated in this study. The results were analyzed statistically by analysis of variance. There was a significant difference among the media and the ones that were different were analyzed by using Duncan's Multiple Range Test to compare the means of polysaccharide production. In addition the viscosity of fermented media was also determined.

I. VISCOSITY

The apparent viscosity of fermented media was measured by using the Brookfield Viscometer and expressed in centipoise. After 96 hours fermentation at 26° -28°C the apparent viscosity of the fermented media was determined at room temperature. The data in Table II show that all acid whey media either containing dextrose or treated with lactase demonstrated higher viscosity than the media without dextrose or lactase. It may be concluded that the added dextrose or lactase increased the viscosity of the fermented media. This indicates that Xanthomonas campestris produced polysaccharide in the medium containing dextrose but not from lactose.

TABLE II

THE APPARENT VISCOSITY OF FERMENTED ACID WHEY AND CONTROL MEDIA

Source	Apparent Viscosity in Centipoise												
	Media												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Means[a]	663.33	2.13	7.33	3.26	13.43	3.13	120.66	4.10	192.00	106.66	128.00	335.00	341.66
Coefficient of Variability (Percent)	31.59	38.96	28.37	19.63	31.64	22.36	39.16	10.97	4.68	77.92	45.06	33.80	87.24

[a]Means of 3 replications

In the acid whey media, medium 3, containing all of the ingredients of the control medium produced the highest viscosity of 341.66 centipoises, while the lowest viscosity of 2.13 centipoises was from the medium 2 which was acid whey only. Media 2, 4, 6 and 8 which did not contain dextrose or were not treated with lactase produced lower apparent viscosity than those which contained dextrose or were treated with lactase. Medium 1 which was control medium produced higher apparent viscosity than medium 13 although medium 13 contained all of the ingredients of the control. This may have been caused by excessive nutrients furnished by medium 13 because acid whey contained most of the nutritional requirements of Xanthomonas campestris. Rogovin and co-workers (29) felt that only the necessary quantity of nitrogen present was necessary for optimum polymer production and also aided in the separation of the polysaccharide. The high protein content of whey may have impeded the production of the polymer and thereby reduced the apparent viscosity.

II. POLYSACCHARIDE PRODUCTION

The polysaccharide production of the thirteen media is shown in Table III. The range was from 0 to 1.3271 grams. The control medium produced the most polysaccharide with a range of 0.5868 to 1.3271 grams while the acid whey media produced polysaccharide from 0 to 1.1907 grams. Even though there was visible abundant growth of the microorganism in all the acid whey media, those without dextrose produced no polysaccharide. In preliminary trials the idea of using lactase might solve this problem. The commercial lactase, Maxilact (produced by Enzyme Development

TABLE III

PRODUCTION OF POLYSACCHARIDE OF THE THIRTEEN MEDIA

Replications	Media												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	grams/300 ml												
1	1.3271*	0.**	0.0143	0	0.0220	0	0.1058	0	0.1235	0.1598	0.0789	0.2673	1.1907
2	1.1647	0	0.0104	0	0.0378	0	0.0897	0	0.1339	0.0470	0.1372	0.1607	0.3752
3	0.5868	0	0.0112	0	0.0259	0	0.0779	0	0.2159	0.0931	0.0989	0.3012	0.0596

*Each value is an average of duplicates.

**0 equals less than 0.1 mg.

Corporation, Keyport, New Jersey) was used. According to the technical information on Maxilact 75 mg were used and 75 percent conversion of lactase could be obtained per quart of fresh pasteurized milk held at 4°C for 16 hours. Medium 3 of whey treated with lactase produced only a small amount of polysaccharide (Table III). As the ingredients of the control medium 1 were added to the whey plus the lactase the amount of polysaccharide produced increased. However, these lactase treated media did not compare with medium 13 in which 3 percent dextrose was added. This could have resulted in a poor conversion rate or otther factors which inhibited polysaccharide production.

Medium 13 produced more polysaccharide than the other acid whey media. It appears that medium 13 contained all of the nutritional requirements for polysaccharide production by the Xanthomonas campestris (29). The very great difference in polysaccharide production among the replicates of medium 13 might have been caused by different composition of the milk from which the acid whey was made. Even though the milk from which the whey was made came from the same large herd of cows and one would assume the source fairly constant, during the latter weeks of February and the first week of March a great difference in the feed due to pasture available may play a part in this difference. In the case of media 2, 4, 6 and 8, there was no polysaccharide produced. It appears this was because those media had neither dextrose nor lactase treatment. In acid whey media, the media which contained 3 percent dextrose produced more polysaccharide than the others. Although medium 13 contained all of the ingredients of medium 1 it did not produce an equal amount of

polysaccharide. It might be that the higher protein and carbohydrate concentration of inhibitied polysaccharide production.

In Table IV the means of the replicates showed a great variation. These variations are not explainable from this experiment.

III. MULTIPLE COMPARISON TEST

The data in Table V show the analysis of variance of the multiple comparison test. Since the calculated F value of 7.2054 exceeded the variance ratio of 3.03 (16), there was a significant difference at the 1 percent level among media. This provided evidence that the different media made a detectable difference in the polysaccharide production. Because of the great variations among replicates the statistical analysis gave no significance among the replications.

The means that were significantly different among the media were determined by using Duncan's Multiple Range Test (33). The means of polysaccharide produced were calculated and compared with the significant range at the 5 percent level. The comparisons are shown in Table VI. This showed that medium 1 was different from all others at 5 percent level. In other words, medium 1 was significantly superior to the other twelve media for polysaccharide production. In case of acid whey media, medium 13 was significantly different from all other acid whey media except medium 12. This showed that medium 13 was superior for polysaccharide production to the other acid whey media, except medium 12. There was no significant difference between medium 12 and 13 which means that acid whey contained the nutrients which are furnished by distillers' dried solubles to produce polysaccharide in the control medium.

TABLE IV

MEAN AND COEFFICIENT OF VARIABILITY OF POLYSACCHARIDE PRODUCTION

Source	Media												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Mean[a]	1.0262	0	0.0119	0	0.0285	0	0.0911	0	0.1577	0.0999	0.1050	0.2430	0.5418
Coefficient of Variability (Percent)	37.92	0	17.31	0	28.87	0	15.37	0	32.09	56.76	28.21	30.17	107.71

[a]Means of 3 replications

TABLE V

ANALYSIS OF VARIANCE OF POLYSACCHARIDE PRODUCTION

Source	DF	SS	MS	F-ratio
Total	38	4.1637		
Treatment	12	3.1560	0.2630	7.2054**
Replications	2	0.1298	0.0649	1.7780^{ns}
Residual error	24	0.8779	0.0365	

**1 percent level significance

^{ns}No significance

TABLE VI

COMPARISON OF THE MEANS OF POLYSACCHARIDE PRODUCTION BY DUNCAN'S MULTIPLE RANGE TEST

	Media												
Source	2	4	6	8	3	5	7	10	11	9	12	13	1
Means	0^{a}	0^{a}	0^{a}	0^{a}	0.0119^{a}	0.0285^{a}	0.0911^{a}	0.0999^{a}	0.1050^{a}	0.1577^{a}	0.2430^{ab}	0.5418^{b}	1.0262^{c}

Numbers denoted by the same letters are not significantly different at 5 percent confidence level.

CHAPTER V

SUMMARY AND CONCLUSIONS

Polysaccharide production in one control and twelve acid whey media was evaluated in this study. The results were analyzed statistically by analysis of variance. There was a significant difference among the media in polysaccharide production as determined by the Duncan's Multiple Range Test.

From the data, the general conclusions in this study may be summarized in the following statements:

1. Xanthomonas campestris grew abundantly in the neutralized acid whey indicating the acid whey contained the necessary nutritional requirements of this microorganism.

2. There was no polysaccharide production in those acid whey media without dextrose or treated with lactase.

3. Acid whey media which contained 3 percent dextrose produced more polysaccharide than those which were treated with lactase.

4. There was no significant difference among replications.

5. There was a significant difference at 1 percent level among the thirteen media for polysaccharide production. Medium 1 was significantly different from all others at 5 percent level. In acid whey media medium 13 there was a significant difference from all other acid whey media except medium 12. In other words production of polysaccharide in medium 13 was superior to the other acid whey media, except medium 12.

Additional research is needed in order to delineate those characteristics of acid whey for polysaccharide production. Some items which need to be evaluated would include dilution of the nutrients in whey, the removal of the whey proteins and adding back a controlled amount and evaluation of lactase activity on acid whey.

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VITA

The author was born on December 17, 1944, in Pei-Kang, Taiwan, Republic of China. He was graduated from Pei-Kang High School in 1963. In 1964, he entered Taiwan Provincial College of Marine and Oceanic Technology and recieved his Bachelor of Science degree in Fishery Technology in 1968. After graduation, he taught at Su-Ao Fishery Vocational High School from 1969 to 1973.

In September 1974, he enrolled in the Department of Food Technology and Science of the University of Tennessee, Knoxville. Since that time, he has been working to complete the requirements for the degree of Master of Science.

He is a member of the Institute of Food Technologists, the professional society for food technologists; and a member of Gamma Sigma Delta, the Honor Society of Agriculture.