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Effect of Chitosan as a Stabilizer in Hot Sauce

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EFFECT OF CHITOSAN AS A STABILIZER IN HOT SAUCE

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ABSTRACT

By the use of mild demineralization in the purification of chitin from shrimp shells and optimizing the deacetylation of this chitin, chitosan capable of producing viscosities of 9600 cps were produced. This is substantially higher than the viscosity of the commercial product, whose viscosity is only 1000 cps.

The effectiveness of this high quality chitosan as a stabilizer in hot sauces was then investigated. From preliminary studies using the commercially produced chitosan, it was concluded that the method of incorporating the gum into the hot sauces was very important. Therefore, three methods of adding the chitosan to the hot sauces were examined. These methods mainly differed in the sequence in which chitosan and acetic acid were combined in order to form a viscous solution. Results indicated that if the chitosan was mixed with the solid components of the hot sauce before the acid, no viscous solutions were formed. Apparently the high salt content (over 5%) of the hot sauces nullifies the viscosity producing potential of chitosan. If the viscous solution was produced before it was mixed with the solids, the two components repelled each other and the solids sedimented to the bottom of the bottle.

INTRODUCTION

Louisiana hot sauce is made from hot red peppers and used quite extensively for the seasoning of many foods due to its piquant flavor. Hot sauce production represents a nine to ten million dollar industry in Louisiana and is one of the most important processed products in the state (Dauzat and Law, 1976). Although the produce may differ somewhat with each processor, the sauce is basically manufactured by aging ground peppers with salt and blending this mixture with vinegar. The main difference among the brands is their consistency, which is due to the size and amounts of the insoluble particles left in the sauce after processing (Noorbakhsh, 1976).

One noticeable problem associated with this product is its tendency to separate into a top liquid phase and a sedimentary bottom portion. This defect in quality of the sauce does not present any toxic effects, however, it is believed to have a negative influence on consumer acceptance. The industry presently adds a commercial stabilizer, xanthan gum, to the sauce which increases its viscosity and prevents the sedimentation of the solid particles. Although this gum is considered to be reasonably effective, it sells for an excess of $4.00 a pound and consequently contributes to

the cost of hot sauce production.

Chitosan is a cationic polymer obtained from the shells of crustaceans, and has the capability of producing viscous solutions when dissolved in certain acidic systems. One of the acids in which chitosan is soluble is acetic. Other industrial gums have the ability to form viscous solutions in water, however, acids reduce their thickening properties by fragmentation of their chain lengths. The viscosity of chitosan is greatest at pH 3 to 4. Because of this property, there is a possibility of a stabilizing effect in hot sauce, which has a high acetic acid content.

This investigation was designed to determine the effect of using chitosan in preventing separation in hot sauce.

REVIEW OF LITERATURE

Structure and occurrence of chitin

Chitin is one of the more important natural structural substances (Giles et al., 1958) and is the second most abundant organic compound on earth (Ruiz-Herrera, 1977). It has long been chemically defined as a high molecular weight polymer of B-D-(1-4) linked N-acetyl-2-amino-2-deoxy-D-glucose units (Fig. 1). It is considered a derivative of cellulose where the C-2 hydroxyl groups have been replaced by acetamido groups. Chitin is often discussed along with cellulose, since it frequently replaces cellulose as the structural entity in the cell walls of many species of lower plants (Ward and Seib, 1971).

Chitin is present in phylas of lower plants and of lower animals. Chitin occurs in the mycelia and spores of many fungi, green algae, and several species of brown and red algae (Ward and Seib, 1971). It has not been found in bacteria, true yeast, and the Actinoymcetes (McNeely, 1959). Chitin is a principle component of the exoskeleton of many lower animals such as in the phylas Annelida (segmented worms), Mollusca, and Arthropoda (Foster and Weber, 1960). Richards (1951) contends that chitin is present in every species of the phylum Arthropoda, which includes the classes

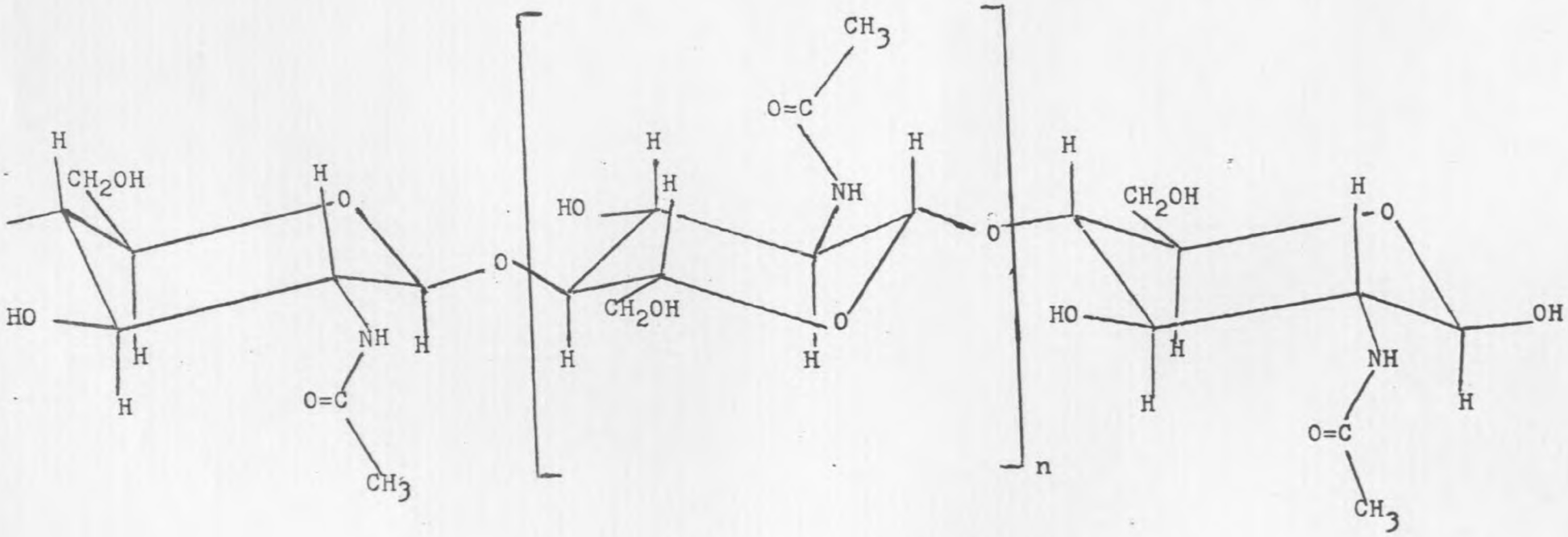

Fig. 1-The structure of chitin.

Arachnida (spiders), Crustacea (shellfish), and the Insecta.

Although chitin is generally considered to occur in Nature as a polymer of completely N-acetylglucosamine units, researchers now agree that the carbohydrate almost always contains some glucosamine units which are not acetylated. Originally, tests showed that there were some free amine groups in the purified chitin, however, this was believed to be the result of degradation during the harsh means of purification, which does indeed degrade the moleucule. Giles et al. (1958) has shown, by means of milder purification techniques, that the structure of chitin consists of 82.5% N-acetylglucosamine, 12.5% glucosamine, and 5.0% water. This corresponds to one-seventh of the monomer units having a free amine group (Muzzarelli, 1973 and Rudall, 1963). Waterhouse and Hackman (1961), by the use of chitinase enzyme treatment, found glucosamine present in quantities less than ten percent of the acetylated residues. Although there is lack of agreement to the exact extent of glucosamine units in "native" chitin, there is no question that the polymer does not consists of completely N-acetylglucosamine units. Difficulties of assessing the exact percentage of deacetylation in the chitin structure, are undoubtedly related to differences in the method of purification and that various species contain different types of chitin (Aveback, 1975 and 1977). Researchers (Hackman, 1962 and

Waterhouse and Hackman, 1961) have found chitin deacetylase activity to vary considerably according to the species examined.

Chitin may differ according to the species from which it is isolated from not only by the ratio of acetylated to non-acetylated glucosamine units, but also by the stereochemical arrangement of its chains. X-ray diffraction investigation (Darmon and Rudall, 1950; Marchesault and Sarko, 1967) have shown that chitin contains highly ordered regions giving one of several possible polymorphic conformations. These crystallagraphic patterns have been designated ∝-chitin, B-chitin, and ϑ-chitin.

Detailed structural work suggests that chains of chitin are arranged in bonded "piles" or "sheets" linked intermolecularly by N-H...O=C hydrogen bonds. The chains of ∝-chitin are of anti-parallel alignment: that is, they are piled on one another with each chain alternating in direction (Carlstrom, 1962). In B-chitin, the neighboring chains are of parallel arrangement, with the reducing sugar of the polymers on the same end of the sheets (Blackwell, 1969 and Dweltz, 1961). The structure of ϑ-chitin is much less understood, however, Rudall (1963) has suggested that the piles of the chains are arranged in sets of three, with two parallel polymers and one antiparallel.

Although three stereochemically different chitin conformations exist, ∝-chitin is by far the most predominate (Hackman and Goldberg, 1965 and Rudall, 1963). The latest model of ∝-chitin includes the following characteristics (Ward and Seib, 1971).

1. polymeric chains in the "bent" conformation.
2. intramolecular hydrogen bonding between a C-3 hydroxyl group and the ring oxygen of the adjacent pyranose ring.
3. full intermolecular hydrogen bonding involving the C=O...H-N groups, and
4. no free OH or NH group and no C=O...H-O bonds in ∝-chitin crystall.

The chitin molecule is known to be of considerable size, but there are many conflicting reports on the actual chain length. Whistler (1973a) states that the molecular weight is 143,000 to 210,000. Bough (1975) indicates that the polysaccharide consists of 2000 to 3000 units, which corresponds to a molecular weight of 406,000 to 609,000. McNeely (1959) reports that deacetylated chitin has a molecule weight of 200,000. This would mean that the original chitin molecule was considerably larger since it is well established that the deacetylation process fragments the polymer (Horton and Lineback, 1965).

Disagreement about the chain length of chitin (i.e. molecular weight) is the result of two main factors:

1. The molecular weight of the chitin will vary according to the method of purification. Milder isolation techniques would of course yield a larger and less degraded polymer (BeMiller, 1965).

2. There may be some derivation in polymer size depending on the species from which it was obtained (Aveback, 1977).

Nevertheless, the molecular weight of chitin is generally considered to be of the same magnitude as that of cellulose (Ward and Seib, 1971).

Preparation of Chitin

Although it is widely distributed in Nature in both the plant and animal kingdoms, the only economical source of chitin at present are the crustaceans, which are gathered on a large scale for food use. Here, large amounts of shells are available as by-products of the freezing and canning industry of shrimp, crabs, and lobsters (McNeely, 1959).

Chitin does not occur alone in the exoskeletons of these invertebrates, but is complexed in a system of calcium carbonate and protein. The amount of $CaCO_3$ in the shell is related to its hardness. Hard shells, such as those of many species of crabs, contain as much as 75% of $CaCO_3$ on a dry weight basis and 15-20% chitin. Softer shelled crustaceans, such as shrimp, contain only 30-40% of the encrusting $CaCO_3$ and as much as 25-30% of chitin (McNeely, 1959). Peniston and Johnson (1970) have recorded yields of 35-40%

chitin from shrimp shells.

The isolation of chitin from crustacean shells is difficult and requires the employment of harsh procedures. Preparation of pure chitin from raw seafood wastes entails a deproteination step by treatment with hot alkali and demineralization by acid treatment. The exact procedure and concentration of reactants may vary, according to the degree purity desired and degradation allowable.

Hot dilute sodium hydroxide is used to remove the proteinaeous material from the shell. Generally, the process consists of adding five to ten percent NaOH at 90-100^{o}C for eight-ten hours. This supposedly extracts the protein without effecting the chitin, however, it has been shown that there is some hydrolysis of the amine groups with the liberation of acetic acid. The deacetylation during this treatment has been estimated at 1.25% (Tracey, 1957) and is a minor concern since the recovered material is usually deacetylated to a greater extent in the preparation of chitosan.

Extraction of the inorganic moiety by the use of acid must be rigidly controlled. Hydrochloric acid removes the calcium carbonate by the reaction:

$$CaCO_3 + 2HCl \longrightarrow H_2O + CO_2 + CaCl_2$$

At the same time, aqueous acids may break the chain of the chitin molecule at the glycosidic linkage between the C-1 and the ring oxygen. In order to protect the chain length of the chitin polymer, the acid must not be very concentrated or very hot. Clark and Smith (1936) report that chitin in 2 N HCl at 100°C undergoes hydrolytic cleaveage of the glycosidic linkages, and at a slower rate, there is also hydrolysis of the amide and amino bonds. Concentrated HCl causes extensive degradation of the chitin molecule, even at room temperature. X-ray defraction studies have shown that there is hydrolysis of the glycosidic bonds within a seventy-five minutes in a solution of concentrated acid and concurrently but more slowly the amide linkages are also broken. Hot concentrated acid completely degrades the polysaccharide into glucosamine and acetic acid (Hackman, 1955).

In order to obtain a polysaccharide with minimal degradation, yet relatively pure of $CaCO_3$, most researchers suggest 5-10% HCl at room temperature for 24 hours or less. With this method of purification, the chitin product has an ash content of 0.5% or less and is essentially devoid of protein (Blumberg et al., 1951). However, even this procedure is considered fairly abrassive and there is obviously some depolymerization of the polymer. According to Richards (1951), pure undegraded chitin has never been

isolated.

The quality of the chitin is reported to be the same whether the alkali or acid treatment is applied first (Blumberg et al., 1951). However, the deproteination step is usually carried out first so that a less degraded protein may be recovered. The protein obtained can be used for animal feeds, which further enhances the economical feasibility of chitin production (Percerval, 1977).

Properties of chitin

Macroscopically, chitin is a leathery amorphous solid that is insoluble in water, dilute acids, organic solvents, and alkalis, but does dissolve in concentrated mineral acids due to depolymerization (McNeely, 1959). Chitin may also be dissolved in hot concentrated aqueous solutions of certain neutral salts, such as LiCNS and $Ca(CNS)_2$, which are capable of a high degree of hydration, but which also fragment the polymer (Muzzarelli, 1973).

Chitin's insolubility in typical solvents is explained by steric conditions that allow close packing of the chains and are held together by intramolecular and intermolecular hydrogen bonding. Water cannot penetrate the crystalline regions and, therefore, cannot bind to the polymer enough to solvate the substance. This results in the material's resistance to dispersion (Hackman, 1955).

Chitosan

Due to chitin's solubility only in solvents which extensively degrade the polymer, aqueous chitin offers little application for industrial purposes. However, chitin can be chemically modified by treatment of hot concentrated alkali, which removes most of the acetyl groups and renders the material soluble in dilute organic acids. This deacetylated chitin, termed chitosan, is capable of producing solutions of high viscosity and sometimes gelation (Whistler, 1973a).

Chitosan is not a definite chemical, but a collective term of various molecules differing in degrees of deacetylation and chain length (Filar and Wirick, 1977), which in turn, governs its behavior in solution. The chemical constitution of chitosan depends on the method of isolation of chitin from the raw material, and the extent of the deacetylation process. Chitin purified under mild conditions and deacetylated to a minimal extent, yields a less degraded molecule.

Chitin's acetyl groups are very difficult to remove due to the trans configuration of the acetamido on the C-2 and the hydroxyl on the C-3 of glucosamine (Wolform et al., 1958). Because of this, it is necessary to employ drastic conditions for deacetylation (Horton and Lineback, 1965).

In the presence of hot alkali, carbohydrates are very susceptible to oxidative degradation (Pigman, 1957; Whistler and Smart, 1953). The hydroxyl groups are the sites most susceptible to attack. Most oxidations are random processes and lead to the introduction of carbonyl and carboxyl groups at various positions in the D-glucose residues (Ward and Sieb, 1971). The presence of the N-acetyl group helps to stablize the chitin molecule, so that it undergoes little, if any, autoxidative degradation in hot dilute sodium hydroxide (BeMiller and Whistler, 1962). The deacetylation process, however, requires concentrated hot alkali and the polysaccharide must be protected against these deteriorative effects by essentially eliminating oxygen from the reaction vessel.

Deacetylation processes may vary according to the concentration of alkali, the temperature of the reaction, and the length of time of heating. Higher temperatures, stronger alkali concentrations, and/or increased time of the reaction increases the degree of deacetylation, and to some extent, also reduces the chain length of the polymer (McNeely, 1959). By controlling the conditions of deacetylation, chitin may be produced in a wide range of viscosity grades. The optimum conditions of hydrolysis depends on the characteristics desired of the final product (Peniston and Johnson, 1970; Wu and Bough, 1977).

Rigby's patent (1936) on chitosan preparation, presented a treatment of chitin with forty percent aqueous sodium hydroxide, for four hours at 110°C in the absence of oxygen. This produced a chitosan capable of producing highly viscous acidic solutions, which indicates a polymer of high molecular weight. Horowitz et al. (1957) was able to almost completely deacetylate chitin by fusion with solid potassium hydroxide at 180°C for thirty minutes while stirring under an atmosphere of nitrogen. After dialysis, the chitogan's chain length was found to be only about twenty units. Fifty percent NaOH for thirty-five to forty hours at 100°C produced a product that contained only six percent acetyl content, but the viscosity of the solution was very low (Tracey, 1957). The minimum practicable concentration of sodium hydroxide is approximately thirty percent, and even this concentration requires thirty to forty hours at 120°C to produce a soluble chitin (McNeely, 1959).

Properties of chitosan

According to Filar and Wirick (1977), the exact degree of deacetylation required to produce a soluble polymer is not readily determined and undoubtedly varies with the molecular weight of the polymer and the nature and concentration of the acid used. Although hard to define, it appears that seventy-five percent free amine is the minimum that will guarantee complete dissolution in most

dilute aqueous systems. As the percent free amine increases, the ease of polymer dispersion increases.

At eighty percent or more primary amine groups, a one percent concentration of chitosan is soluble in a one percent concentration of acetic, adipic, formic, lactic, malic, malonic, propionic, pyruvic, and succinic acids. A one percent chitosan is not soluble in a one percent solution of oxalic, citric, or tartaric acid, but if the acid concentration is raised to ten percent, solubility is achieved (Hercules, Inc., undated).

In acidic media, chitosan functions as a cationic colloid because of the basicity of the free amino groups (Filar and Wirick, 1977; Muzzarelli, 1973). When the amino group picks up the positive charge ($-NH_3^+$), there is enough repulsion of the chains to disrupt the molecular agglomeration and the chitosan molecules uncoil and assume a more elongated shape. This produces the available hydroxyl binding sites of the polysaccharide which can then bind with water (Whistler, 1973b). This allows the polymer to become solvated, the chains disperse throughout the solution, and the chitosan is dissolved in the liquid.

While the solubility of chitosan is a function of the primary amine groups, the viscosity producing capabilities are a function of the polymer chain length (Levy, 1961). The more chitin is deacetylated and converted to a higher

percentage of free amines, the more its polmer is fragmented and its ability as a viscosity builder reduced.

The molecular weight of chitosan is very hard to elucidate. Shorter chain polymers have been identified by dialysis (Foster et al., 1957); Horton and Lineback, 1965). However, the larger polymers are much more difficult to define. The molecular size may vary according to the manufacturing conditions used and the molecular weight values also depend on the method of its measurement. Viscosity studies generally indicate very large polymer lenght. But according to Wu and Bough (1977), in those cases in which molecular weight values of over one million are observed, the association of monomer units for form large oligomer complexes may be involved. The nature of the forces on bonds that hold the complex together are unknown at present, though are believed to be the result of the polyelectrolyte behavior of the polymer in acidic solutions which causes the molecules to overlap each other producing a larger configuration (Muzzarelli, 1973). Muzzarelli (1973) added sodium formate to a chitosan-formic acid solution to depress the polyelectrolyte effect, and by the use of light scattering techniques extrapolated the molecular weight of a chitosan polymer to be 120,000. Wu and Bough (1977), by the use of high liquid chromatography procedure, found M.W. values for several commercially prepared chitosan samples

to be 110,000 to 3,999,000 even after the polyelectrolyte effect in acetic acid was minimized by sodium acetate. Even though the association of chitosan's chains represent difficulties in defining the molecule's exact size, it causes the material to behave as a high molecular weight polymer and enhances its viscosity producing ability.

Kytex

Chitosan is commercially produced under the tradename, Kytex, a product of Hercules Incorporated, Wilmington, Delaware. Kytex is available in three grades, differing in their viscosity producing ability. One per cent of the high grade product produces a viscosity of at least 1000 cps in a one percent acetic acid solution, and is approximately eighty percent deacetylated. The medium grade Kytex yields a viscosity of 100-250 cps at the same concentration mentioned above, and is estimated at being 82.5% deacetylated. The low grade chitosan produces a solution of 25-50 cps at a concentration of two percent polymer in two percent acetic acid, and is approximately eighty-six percent deacetylated (Filar and Wirick, 1977).

From the data given above, it can be seen that with commercially produced chitosan only a range of viscosities can be assured for a particular grade. High grade Kytex purchased at different times have been found to exhibit

different properties in solution (Filar and Wirick, 1977). Although a one percent concentration of high grade Kytex ix stated to have a minimum viscosity of 1000 cps, the same source recorded the product producing a viscosity of 2760 cps, which is nearly a three fold difference.

Viscosity and chain length

In the solid state, all polysaccharides chains are linked together by intermolecular and intramolecular hydrogen binding. The arrangement of the chains are never entirely packed uniformly, therefore, the hydrogen binding sites are only partially satisfied. The unbound hydrogen binding posititons have a strong affinity for water and avidly hydrate when the polymer is placed in an aqueous medium. If the forces holding together are not as great as their affinity for water, the polysaccharide will be completely solvated until it becomes surrounded by an environment of water (Whistler, 1973b). As the colloid is dispersed and the liquid is absorbed, the solubilized polymer swells resulting in the thickening of the liquid phase. This thickening of the solution decreases its ability to flow, or in other words, the viscosity is increased (Glicksman, 1969). The viscosity capability of a polymer is related to its chain length (Levy, 1961 and Werbin, 1960). The larger the length of the chain, the more space it will occupy in the solution, and its ability to immobilize the solvent will be increased. Scission of the chain is

associated with a decrease in viscosity. The hydrolytic cleavage of polysaccharide glycosidic linkages is strongly influenced by acidic media (Levy, 1961).

High molecular weight chitosan is known to be very stable in solutions of low pH. According to Barker et al. (1958), chitosan was not completely hydrolyzed by treatment with 3.3 N HCl at 100°C for three days. Chitosan's remarkable resistance to acidic hydrolysis is due to its cationic nature in acid solutions (Fig. 4). The positive charges acquired by the basic amino groups in the acid media, electrostatically shields the neighboring glycosidic constituents from attack by hydrions (Foster et al., 1957).

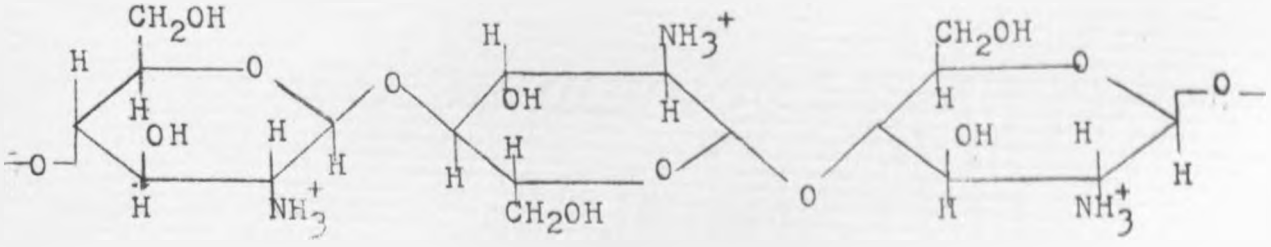

Fig. 2--Cationic chitosan.

Viscosity and stabilization

When solid particles are dispersed uniformly through a continuous liquid phase, the resultant system is called a suspension (Glickman, 1969). Gums, by increasing the

viscosity of a solution, exert a stabilizing influence by acting as a bridge between the continuous phase and the particles which they envelop (Myers, 1960). According to Glickman (1969), the thicker, or more viscous, the liquid phase the slower the solid particles will settle out. Reduction in viscosity may cause the accelerated sedimentation of the formed suspended particles (Werbin, 1960).

Potential of chitosan

It is estimated that 293 million pounds of crab, 29 million pounds of lobster, and 372 million pounds of shrimp were caught in the United States in 1973. This amounts to 700 million pounds of shellfish landed. About 350 million of the harvested crustaceans are produced by food manufacturers. Of this, it is calculated that sixty percent, or 210 pounds, of the total weight is waste (Anon., 1976).

Environmental problems and cost associated with the disposal of shellfish processing wastes have increased. Shellfish processors are no longer able to dump extracted carcasses into waters adjacent to their plants without risking federal restraining action and serious penalties under various environmental laws and regulation (Anon., 1976).

Chitin and its derivatives are potentially profitable by-products that can be recovered from these processing wastes. However, due to the perishability and the large

percentage of other materials in the shell, it appears that large suppliers of chitin must be within a fifty mile radius of the seafood processor for the economic viability of chitin recovery and production (Murray and Hattis, 1977). Only the areas of centralized shellfish harvesting afford the potential for gathering of sufficient quantity to be of commercial feasibility. Even here, more industrial demand must be initiated for the production of chitosan to be of economic incentive. Producing chitosan of high quality is not an inexpensive process and natural variation in raw material adds to the expense of quality control. The recovery and sale of the protein by-products, will help to defray significant portions of production costs (Johnson and Peniston, 1977; Percerval, 1977).

Possible applications

The conceivable markets for chitin and chitosan are very diverse. Speculative applications include chitosan as an adhesive, as an additive in the paper and textile industries, as an absorbent to bind heavy metals for industrial and nuclear plant waste, as a cementing agent for leather manufacture, as a coagulant for recovery of flocculating materials in suspension, and as a viscosity builder of acidic solutions (Anon., 1976 and Muzzarelli, 1973).

Thus far in the food industry, chitosan has been investigated for its effectiveness of treatment of various food processing waste. Bough has shown chitosan to be an effective coagulating agent for reduction of suspended proteins in the processing wastes from vegetable, animal slaughtering, and egg breaking plants (Bough et al., 1975). Recovery of the coagulated by-products obtained by treatment with chitosan has potential for utilization as animal feeds. The effectiveness of chitosan in the recovery of these sludge proteins is based on its positively charged properties. Consequently, for this particular application, it is important to have a polymer with a high percentage of free amine groups, and because of this, a lower viscosity chitosan is used (Bough et al., 1975; Wu and Bough, 1977).

The exploitation of chitosan's viscosity producing ability in the food industry has not yet been recorded in the literature.

Approval of chitosan as a food additive

In order for chitosan to be used as a food additive its safety must first be approved by the Food and Drug Administration.

Carbohydrates, when administered orally, are generally considered to be innocuous (Whistler, 1973b). Nutritionally, they can be classified as being either digestible and

absorbed by the body, or indigestible and disposed by the body through the feces. The monosaccharides of chitosan, as in cellulose, are linked by B-(1 - 4) glycosidic bonds, and therefore, the polymer is not hydrolyzed by human digestive enzymes (Pike and Brown, 1975). The long chain fibers are not absorbed by the human alimentary tract and their dietary significance is only in contributing to bulk in the diet.

In preliminary test trials chitosan, in the form of dried flakes, was fed to rats at 0.0, 0.5, 1.0, 2.0, and 5.0% of their diet. Except for the animals fed the highest amount, 5.0%, the rats grew as well as those fed control diets as indicated by final weight and feed efficiency. The rats were healthy and vigorous and displayed no overt symptoms of physiological distress due to the consumption of chitosan. Hemoglobin and hematocrit levels in the blood were normal (Bough et al., 1975).

The slight weight loss of the animals fed 5.0% chitosan is believed to be due to the chitosan being fed in dry flake form, which effected the palatibility of the feed. When dissolved in dilute acetic acid, these palatibility factors should be overcome.

Hot sauce production

Hot sauce production begins by grinding the peppers of the Capsicum annum or C. fructescens varieties into what is known as pepper mash. The mash is mixed with ten to twenty-five percent salt and aged in wooden barrels for an indefinite time between several months to three years. The aged mash is then blended with vinegar and an edible stabilizer. This mixture may or may not be ground again, but is strained through screening machines which remove the large particles of the skin and seed. The finished product is then bottled, labeled, and marketed. This process is outlined in Fig. 3.

Described above is the general process, however, the exact procedure may vary somewhat and is considered to be a guarded trade secret. The main differences among the brands are the amounts of salt added to the mash, the time the mash is allowed to age, the degree of grinding and straining of the insoluble material, and the amount of vinegar added to the final product. The composition of different brands of hot sauce range between 81 to 95 percent moisture, 3 to 8.5 percent acetic acid, pH's of 2 to 3, and a salt content of 2 to 15 percent (Noorbakhsh, 1976). These factors undoubtedly have a pronounced influence on the separation problem.

The hot sauce industry presently uses the micorbial polysaccharide, xanthan gum, to prevent the sedimentation of the insoluble matter from the liquid phase. A concentration of one percent of xanthan gum yields a viscosity of approximately 1000 cps when measured with a Brookfield Model LVF Viscometer no. 3 spindle at 60 r.p.m. Viscosities of this polymer are said to be essentially unaffected by a pH between 6 and 9, and only show minor variation in a viscosity range of 1 to 11 (McNeely and Kang, 1973). The industry presently uses a concentration of 0.25% xanthan gum in hot sauce to prevent the separation.

Fig. 3--Flow diagram of hot sauce processing (Noorbakhash, 1976).

Extraction of chitin from shrimp shells

Chitin was prepared by extraction from shrimp shells obtained frozen from Reuter's Seafood Co., New Orleans, Louisiana. The shells were stored frozen until needed. When taken from storage, the shells were washed thoroughly under tap water to remove most of the residual meat and other extraneous material. After cleaned, the shells were dried in a fluidized-bed drier for one hour at 60°C.

Two hundred grams of the cleaned and dried shells were extracted with 2 liters of 5% sodium hydroxide (NaOH) and heated to 90°C and held at that temperature for four hours. The NaOH medium was then poured off and the residual material was rinsed with tap water. The collected material was then digested with 2 liters of previously refrigerated 5% hydrochloric acid (HCl) and the mixture maintained at 2°C for twenty-four hours. The HCl solution was then removed and the chitin was thoroughly washed and dried. This flakey material was ground with a waring blender to a coarse powder and stored in plastic jars tightly closed.

Preparation of chitosan

Chitosan was prepared by the deacetylation of two different sources of chitin. The chitin was obtained using

the method described above, and from the L.S.U. Food Science Department (chemical history unknown). Several different times of deacetylation were investigated.

Approximately 40 g of coarsely ground chitin were added to a 1000 ml flat bottom boiling flask. Forty percent NaOH previously heated to 90°C was poured into the flask until the level of the chitin-NaOH mixture was near the top of the body of the flask. The flask was then quickly attached to a Wheaton-Allin reflux condenser and the solution was heated to and maintained at 100-110°C with a Bunsen burner for varying times of 1, 2, 4, 6, and 8 hours. At the completion of the heating process, the mixtures were rinsed of the liquid and washed thoroughly. The last washing consisted of immersing the insoluble material in hot water for several minutes, after which the water was filtered off and the chitosan was dried in a fluidized-bed for one hour at 60°C. The dried chitosan was stored in plastic jars, tightly closed until needed for further use.

Determination of chitosan quality

Nitrogen content--The nigrogen in chitin and chitosan samples were analyzed by the Kjeldahl-Gunning modification of the classic Kjeldahl method (A.O.A.C., 1970). The chitin samples were first treated with hot 5% NaOH to assure the removal of any residual protein, and

then washed and dried. Approximately 1 g samples were accurately weighted to 0.1 mg on weigh paper. The weighed samples were added to the digestion flask with 1 "Kel-pak" No. 2 (containing $CuSO_4$ and K_2SO_4) and 35 ml of sulfuric acid (H_2SO_4). The samples were digested for thirty minutes after the solution turned a clear blue color and allowed to cool on the rack. One hundred seventy-five milliliters of tap water were then added to each flask and allowed to again to cool. Several pieces of mossy zinc were added to the flasks and 140 ml of 50% NaOH were carefully added to the solution by tilting the flask and slowly pouring in the aqueous alkali solution. The Kjeldahl flasks were immediately attached to the distillation stills and were shaken thoroughly. The solutions were distilled into 50 ml of 4% boric acid with 11 drops of methyl red-methylene blue indicator until the level of the distillate reached 225 ml. The contents of the receiving flasks were titrated with 0.1 N HCl until the green solution turned a faint violet color. The percent nitrogen was calculated by the equation:

$$\% \text{ Nitrogen} = \frac{(\text{ml of HCl}) \times (N) \times (14) \times 100)}{(\text{g sample}) \times (1000)}$$

Moisture and ash content--Approximately 2 g samples were accurately weighed in cleaned tared porcelain crucibles. The samples were then dried in an oven at 105°C for twenty-four hours after which they were allowed to cool in a

desicator. The percent moisture was taken as the ratio of weight lost to original weight of the sample times 100. The dried samples were then ashed in a muffle furnace preheated to 600°C and held at 550-600° C for three hours. The crucibles were then allowed to cool to approximately 100°C in the furnace and transferred to a desicator and cooled to room temperature. The ash content was taken as the ratio of the weight of the ash to the weight of the sample times 100, for a percentage.

Determination of viscosity--The viscosity of all chitosans were of 300 ml solutions in a glass container 7 cm in diameter. The dry chitosan was first dispersed in 150 ml of distilled water with a high speed stirrer, and while agitation continued, the acid solution was poured into the vortex. The agitation was maintained until maximum dissolution of the chitosan was obtained, which usually required about 10-15 minutes. Viscosity measurements were always made shortly after the preparation of the sample, and were determined in the same container that was used to prepare the solution to prevent time loss and messiness of transfer.

The viscosity of the solution was measured with a Brookfield Syncho-Lectric Viscometer. The viscometer's spindles were centered in the solution and immersed to the groove on their shafts. The appropriate spindle and speed were used for each viscosity determination, and are stated

with the results.

Preliminary studies using Kytex and xanthan gum

Levels of 0.5, 0.4, 0.3, 0.2, 0.1, and 0.0% (control) commercial chitosan were incorporated into hot sauce to preliminarily test its effectiveness in preventing the sedimentation problem. The chitosan used was High Grade Kytex, produced by Hercules, Inc.. Three hundred grams of fermented pepper mash (obtained from B.F. Trappey's Sons, Inc.) were mixed with a sufficient amount of diluted 120 grain vinegar to make a 600 ml solution. Appropriate amounts of Kytex were added to 400 ml of vinegar (diluted to 1% acid) and mixed in with the pepper mash solution so that the final product was 30% mash and 4.5% acetic acid. The resulting hot sauce samples were then poured into 6 oz bottles and stored in boxes in order to avoid any abrupt movement.

Xanthan gum was also incorporated into the hot sauce at the same concentrations as the Kytex to compare its effectiveness to that of the chitosans. The xanthan gum was first dissolved in water, then mixed with the mash and vinegar so that the concentrations were the same as that used for the chitosan.

Effectiveness of high viscosity chitosan in hot sauce

The chitosan deacetylated for four hours was incorporated into hot sauce because it was capable of producing

the highest viscosity. Three ways of producing the sauce were examined. The mash used was of the same batch for each investigation and was first ground in an industrial waring blender. In each case the hot sauce was made with thirty percent mash and had a final concentration of 4.5% acetic acid.

Method No. One--This method involved first mixing the chitosan powder with the ground mash, and then adding the vinegar to the mash-chitosan mixture in the correct proportions to produce a product of the desired composition.

Five hundred grams of hot sauce were prepared by thoroughly mixing the appropriate amount of chitosan to 150 g of ground pepper mash. Three hundred fifty milliliters of 6.4% acetic acid (prepared by diluting 187 ml of 120 grain vinegar and 163 ml water) were then poured into the mash-chitosan mixture under vigorous agitation. The resulting solution was then strained through a 20 mesh screen and poured into 6 oz bottles which were stored as to prevent any shaking.

Method No. Two--This method of hot sauce production entails dispersing the chitosan in water, and then adding this mixture to the mash in which all the vinegar has been blended together.

One hundred eighty-seven milliliters of 120 grain vinegar were mixed with 150 g ground pepper mash until the

mixture was homogenous. With agitation of a high speed stirrer, the chitosan powder was dispersed in 163 ml of water and then added to the mash-vinegar mixture while the agitation was continued. The resulting sauce was strained through a 20 mesh screen and poured into bottles and stored in boxes.

Method No. Three--This method of hot sauce production is exactly the same as that which was used in the preliminary investigation except that the chitosan capable of producing the highest viscosity was used.

Evaluation of the stabilizing effectiveness

The degree of separation of the hot sauces was assessed by a numerical grading system. The correlation between numbers and description is as follows:

No. 1 ---- no separation

No. 2 ---- slight to hairline separation

No. 3 ---- moderate separation

No. 4 ---- considerable separation

No. 5 ---- extensive separation

No. 6 ---- gross separation

The standard characteristics that correspond with each of these descriptions are shown in Fig. 4.

No. 1 No. 2 No. 3 No.4 No. 5 No. 6

Fig. 4--The standards used in grading the separation of the hot sauces.

Determination of salt

Approximately 2 g of pepper sauce sample were accurately weighed into a 250 ml Erlenmeyer flask. One hundred milliliters of boiling water were added, and the solution was allowed to cool for approximately ten minutes with occasional stirring. A ten milliliter aliquot was then pipetted into another Erlenmeyer flask in which 2 ml potassium chromate indicator were also added. This solution was diluted with water and titrated with 0.1 N silver nitrate ($AgNO_3$) until an orange-brown color persisted for thirty seconds. The concentration of salt was calculated by:

$$\% \text{ NaCl} = \frac{\text{ml } 0.1 \text{ N } AgNO_3 \times 5.85}{\text{g sample}}$$

RESULTS AND DISCUSSION

A preliminary test was designed to compare the stabilizing effectiveness of a commercially produced chitosan, High Grade Kytex, to that of the present stabilizer, xanthan gum. Levels of 0.1, 0.2, 0.3, 0.4, and 0.5% of each gum were used.

For the incorporation of the Kytex, the polymer was first dissolved in one percent acetic acid (diluted vinegar). This viscous solution was then blended with a mash-vinegar mixture to produce a hot sauce of 4.5% acid. With this method of producing the sauce, obtaining a uniform product was a major problem and most difficult with the higher concentrations of chitosan, even after vigorous agitation.

The xanthan gum was incorporated by first dissolving the gum in water, and then adding it to the mash and vinegar to yield a hot sauce of the same acid and mash content as the Kytex's. Although agitation was required, this product was more homogeneous than the chitosan prepared hot sauce. The results of this study are shown in Figs. 5 and 6.

At levels of 0.3% and above, the xanthan gum was very effective in preventing separation of the sauce. At a concentration of 0.2% some stabilization was displayed, but at 0.1% the colloid offered very little advantage over the control sample.

Control 0.1 0.2 0.3 0.4 0.5

Fig. 5--High Grade Kytex in hot sauce at various levels.

Control 0.1 0.2 0.3 0.4 0.5

Fig. 6--Xanthan gum in hot sauce in various levels.

The effect of the Kytex was less favorable. With concentrations of 0.1 and 0.2%. the Kytex was comparable to that of xanthan gum. The higher levels, though, were no more effective than the 0.2%. It was also apparent that at the higher concentrations (0.4 and 0.5%), the nature of the chitosan prepared hot sauce was different from that of the xanthan gum product. The suspension media appears to have congealed phases. This is probably due to the difficulty in obtaining a completely uniform mixture. The evaluation of the separation of the hot sauces at the various concentrations of Kytex and xanthan gum are given in Fig. 7. At this point in the investigation it was concluded that when Kytex was utilized in this manner, it did not adequately prevent the separation of hot sauces.

Two strategies were used in an attempt to increase the stabilizing effect of chitosan. First, a higher quality chitosan (expressed by viscosity) was attempted to be produced, with the assumption that it would require smaller quantities to produce the same viscosity of a poorer quality chitosan. Smaller concentrations of chitosan should be easier to disperse, and thus a more homogeneous mixture should be obtained. Secondly, the method of incorporation was investigated. The stabilization potential might be increased if the gum was incorporated by a different method.

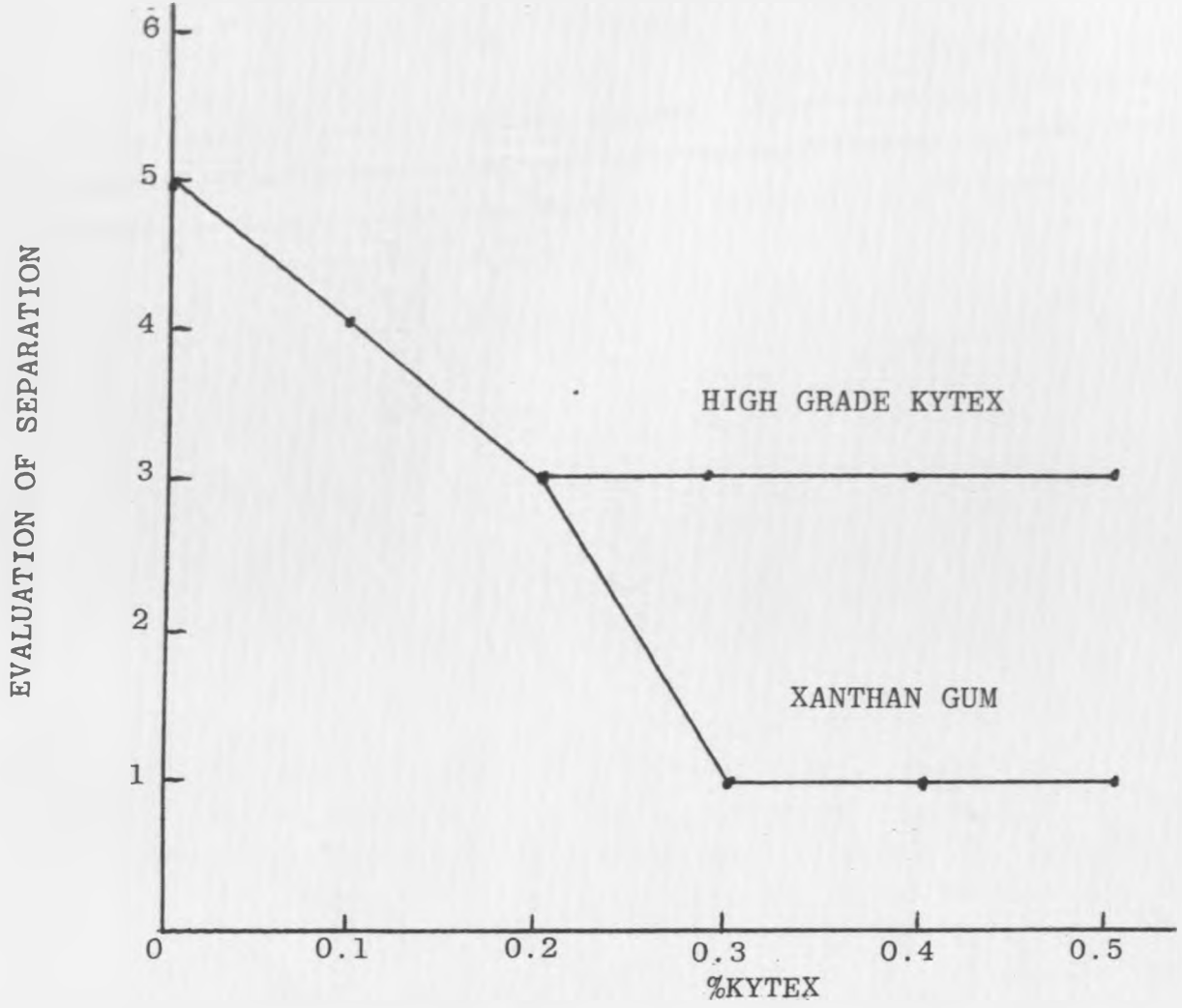

Fig. 7--The evaluation of the separation of hot sauce using different levels of High Grade Kytex and xanthan gum.

Production of a chitosan capable of higher viscosity then the commercial brand was investigated by optimizing the time of deacetylation treatment. Chitin must be deacetylated with concentrated hot alkali to free its amino groups so that it could become soluble in aqueous acid systems. This basic free amine group picks up a positive charge from the protonation of the acid. The acquired positive charge results in the repulsion of the entangled chains of the polymer, so that the molecules uncoil and the chitosan is solubilized. The exact amount of free amines required for a soluble polymer is not easy to pinpoint and varies with different chitosan samples. Filar reports (1977) that the amount of deacetylation is usually about seventy-five percent but depends on such factors as the acidic solvent used and the exact configuration of the chitosan molecules.

The extent the polymer is deacetylated is related to its nitrogen content, as shown in Fig. 8. Chitin completely deacetylated chitin is 8.7% nitrogen. Analysis of commercial chitin and three grades of chitosan are revealed in Table I.

The estimated percent deacetylation is extrapolated from Fig. 8. These results indicate that a decrease in viscosity is accompanied with an increase of deacetylation of the soluble polymers. The high grade chitosan (1180 cps) had the lowest amount of acetyl groups removed, whereas the lower grade (75 cps) had the greatest amount of deacetylation.

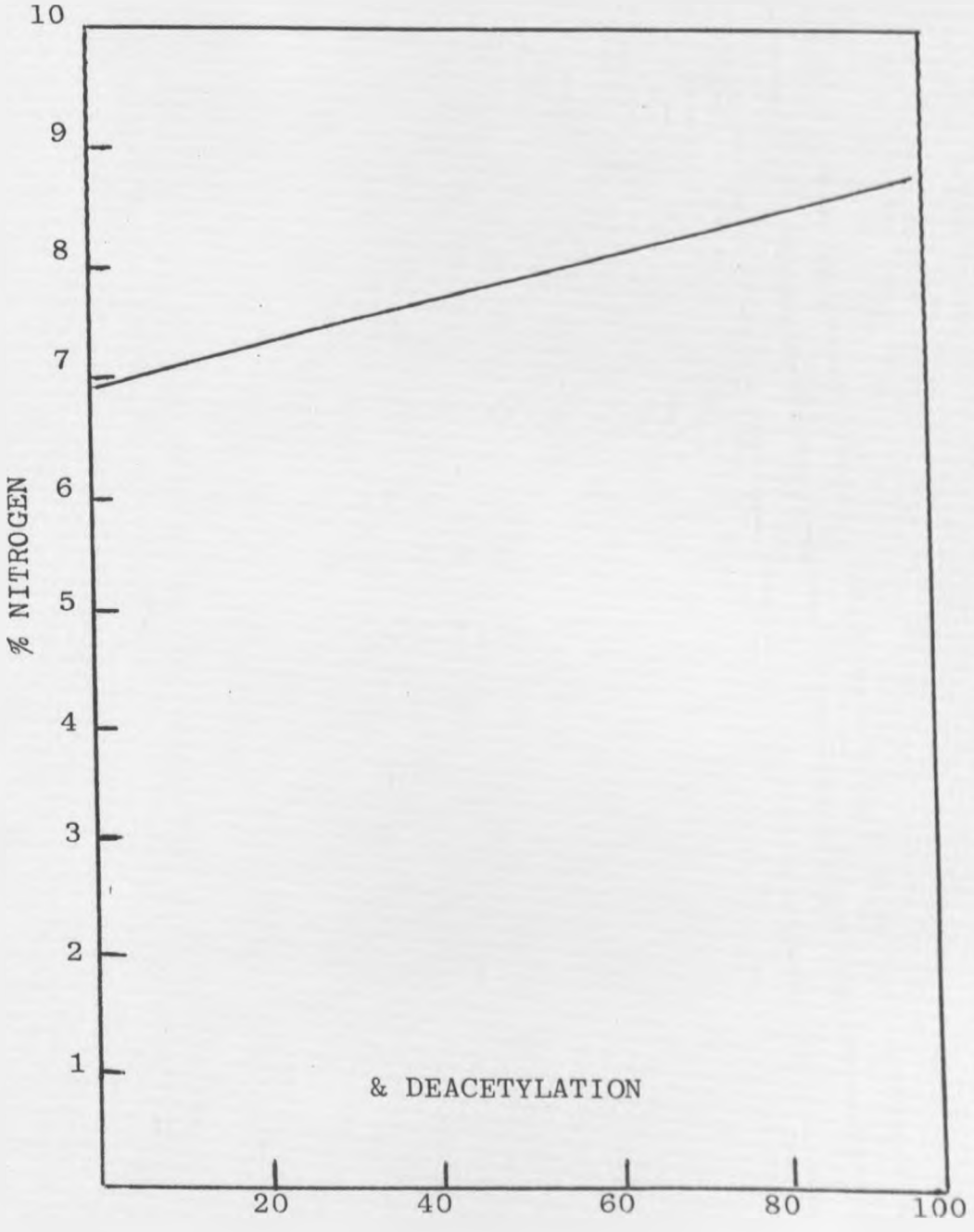

Fig. 8--Relationship between % nitrogen and % deacetylation.

TABLE I

Composition and Viscosity of Commercially Produced Chitosans

Sample	% Ash	% Moisture	% N[(a)]	Est. % deacetylation	Viscosity(cps)
Chitin	0.82	9.01	6.82	--	Insoluble
High Grade Chitosan	1.02	9.67	8.32	79	1180[(b)]
Medium Grade Chitosan	0.62	10.32	8.36	81	115[(c)]
Low Grade Chitosan	0.89	8.08	8.47	87	85[(d)]

(a) on an ash and mositure free basis

(b) 1% polymer in 1% acetic acid determined with spindle no. 3 at 30 r.p.m.

(c) 1% polymer in 1% acetic acid determined with spindle no. 2 at 30 r.p.m.

(d) 2% polymer in 2% acetic acid determined with spindle no. 1 at 30 r.p.m.

A chitosan product which has a higher nitrogen content has undergone a more extensive (and degradative) deacetylation process. A high quality chitosan, therefore, should be deacetylated just to the threshold of solubility, with as little of the abrassive deacetylation treatment as possible.

Chitin (chemical history unknown) was obtained from the L.S.U. Food Science Department, and was deacetylated for 1, 2, 4, 6, and 8 hours with 40% NaOH at 100-110^{o}C to elucidate which conditions would produce the most viscous chitosan. Table II shows the analysis of the products of these treatments.

These results indicate that deacetylation with hot 40% NaOH for one hour produced a polymer with only twenty-nine percent of the acetyl groups removed and did not yield a soluble chitosan. The two hour process, however, produced a soluble chitosan with only forty-four percent deacetylation, but the viscosity was only sixty-nine cps. Since the chitosan was soluble with only forty-four percent removed and it produced a very low viscosity solution, it was concluded that polymer chains must have been severely degraded in the chitin purification.

In order to produce a viscous chitosan, chitin was extracted from shrimp shells under very mild conditions. Five percent NaOH at 90^{o}C was used to remove the protein moiety of the hulls. This treatment is assumed to be

TABLE II

Composition and Viscosity of Chitosan Produced From a Chitin with Unknown Chemical History

Sample	% Ash	% Moisture	% N[(a)]	% Deacetylation	Viscosity(cps)[(b)]
Chitin	1.35	10.55	7.01	--	Insoluble
1 hr. Chitosan	0.96	8.98	7.41	29	Insoluble
2 hr. Chitosan	1.12	9.61	7.69	44	69
4 hr. Chitosan	0.85	9.64	8.30	78	61
6 hr. Chitosan	0.61	8.09	8.30	82	63
8 hr. Chitosan	0.79	7.21	8.41	84	53

(a) moisture and ash free basis

(b) 1% polymer in 1% acetic acid

harmless to the structure of the chitin, since hot concentrated alkali is required for the deacetylation process. The demineralization step, however, is very important. Either concentrated or hot dilute HCl is reported to hydrolyze the B-glycosidic linkages of chitosan (Clark and Smith, 1936; Hackman, 1962) to various degrees. For this reason, cold dilute acid was used. When the acid and deproteinized shells were combined and stirred, the mixture immediately began to froth which indicates the conditions were strong enough to remove the $CaCO_3$. Analysis of the sample showed an ash content of 1.53% which further indicates an acceptable demineralization step.

The chitin obtained by this method was then deacetylated with forty percent NaOH at 100-110^{o}C for 1, 2, 4, 6, and 8 hours. The composition of these samples are given in Table III.

The chitin deacetylated for one hour had an acetyl content of thirty-four percent and was insoluble. The processed chitin had a lower viscosity (8600 cps) than the four hour sample (9600 cps). Since the polymer with the minimal amount of deacetylation to permit solubilization should be the most viscous, it is concluded that the two hour chitosan only partially solubilized and, therefore, all of the sample did not contribute to the viscosity. The four hour chitosan had been deacetylated sufficiently to

TABLE III

Composition and Viscosity of Chitosan Produced from Minimally Degradated Chitin

Sample	% Ash	% Mositure	% N[a]	Est. % Deacetylation	Viscosity(cps)[b]
Chitin	1.53	8.41	6.81	----	Insoluble
1 hr. Chitosan	0.98	10.02	7.51	34.0	Insoluble
2 hr. Chitosan	1.08	7.49	7.84	54.0	8600
4 hr. Chitosan	0.79	9.03	8.17	70.5	9600
6 hr. Chitosan	0.91	8.89	8.27	76.0	7700
8 hr. Chitosan	0.82	7.80	8.31	78.5	1400

(a) moisture free baiss

(b) 1% polymer in 1% acetic acid measured with spindle no. 4 at 30 r.p.m.

yield a more soluble polymer, yet not enough to reduce its viscosity potential.

Chitin deacetylated for six hours showed only a slight decrease in viscosity (7700 cps) from the four hour sample, which indicates the rate of deacetylation is sharply reduced. As the process continues, fragmentation of the polymer increases as shown by the viscosity of the eight hour product (1400 cps).

Since the chitosan deacetylated for four hours exhibited the most viscous solution, it was chosen as the sample to be further tested as the stabilizer in hot sauce. Comparison of the viscosities of this product and High Grade Kytex at various levels in 4.5% acetic acid is given in Fig. 9.

Three methods of incorporation with the high quality chitosan were investigated. Lower concentrations of the colloid were examined than in the preliminary study with Kytex, because lower levels are needed to exhibit the same viscosity. For an accurate comparison of the three methods, the mash used was from the same batch, since different batches may behave differently in regards to their tendency to separate.

The first method of hot sauce production involves blending the ground mash and dry chitosan first. After the gum is thoroughly mixed with the pepper's solids, the

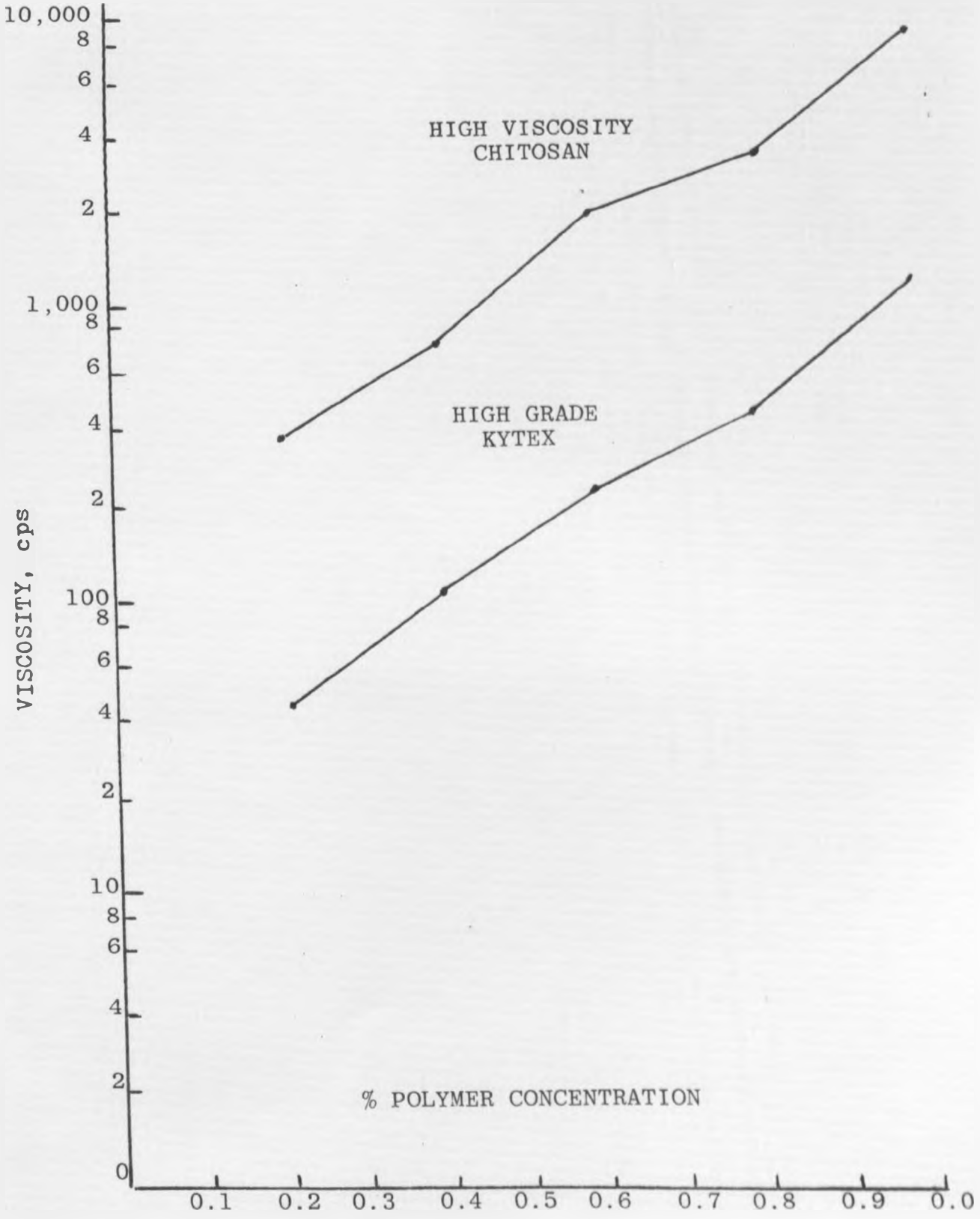

Fig. 9--Comparison of High Grade Kytex to high viscosity chitosan in 4.5% acetic acid at various concentrations.

vinegar is added, so that the polymer swells after it is dispersed throughout the medium.

In the second study, chitosan was first dispersed in water to wet the dry material, then mixed with the mash after all the vinegar had been added. Here, the chitosan would swell gradually, as it was added to the solids and acetic acid.

Thirdly, the hot sauce was produced with the high quality chitosan in the same procedure as used with the Kytex. With this method, the chitosan has formed a viscous solution before it is blended with the rest of the components. The result of the three methods are given in Fig. 10, 11, and 12, and assessed in Fig. 13.

These results show that even with the high viscosity of chitosan, the sauces had a tendency to separate in methods 1 and 2 of incorporation. As depicted in Fig. 10, the chitosan offered no advantage in preventing the separation at levels of 0.025 and 0.05%. With 0.1% chitosan there seems to be some favorable influence, although some separation is evident. At levels of 0.2 and 0.3% the separation is actually greater than the control. It appears that at these higher levels the chitosan not only was ineffective as a stabilizer, but also produced an adverse effect.

The results shown in Fig. 11 illustrate almost

Control 0.025 0.05 0.1 0.2 0.3

Fig. 10--High grade chitosan incorporated into hot sauces by method 1.

Control 0.025 0.05 0.1 0.2 0.3

Fig. 11--High grade chitosan incorporated into hot sauces by method 2.

Control 0.025 0.05 0.1 0.2 0.3

Fig. 12--High grade chitosan incorporated into hot sauce by method 3.

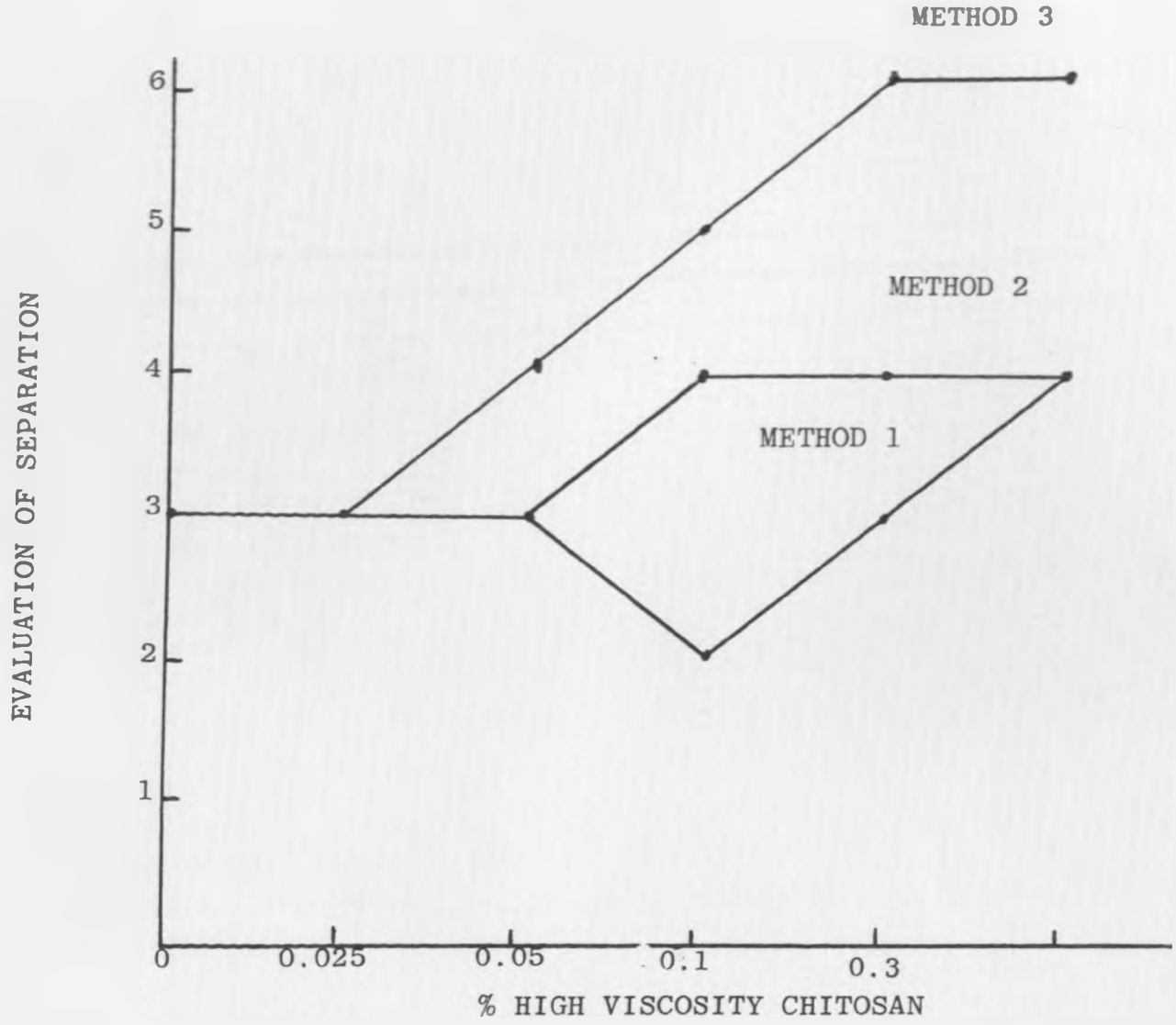

Fig. 13--The evaluation of the separation of hot sauce using different levels of high viscosity chitosan.

identical effects. Lower concentrations of the chitosan exhibited no stabilizing influence, whereas the higher levels increased the amount of separation.

Rheological measurements of the hot sauces produced by methods 1 and 2, indicated that the viscosities of the product did not increase as the levels of chitosan were raised. It was theorized that the high salt content of the sauce effected the viscosity of the chitosan. The salt content of most hot sauces have been reported to be 4.75 to 15.18% (Noorbakhsh, 1976). The salt content of the hot sauce in this investigation was found to be 5.53%. An experiment was then designed to examine the effect that this level of salt would have on the viscosity of chitosan.

Chitosan at levels of 0.1, 0.2, 0.3, 0.4, and 0.5% were mixed with 5.5% salt. Water and 120 grain vinegar were then mixed in with vigorous agitation. In all cases the chitosan never went into solution and, therefore, no viscous solutions were formed. To go into solution, the amine groups of chitosan must obtain a positive charge from the acidic environment. The salt increases the ionic density of the liquid and the equilibrium $-NH_2 + H^+ \rightleftharpoons -NH_3^+$ is driven to the left. Consequently, the chitosan's chains do not repel each other and do not go into solution.

The outcome of the third method of incorporation was vastly different than that observed in methods 1 and 2.

Although the 0.025% chitosan displayed no effects different from the control, as the concentration of chitosan increased it gradually augmented the separation of the solids. Since the chitosan was first dissolved in the vinegar, its amine groups were able to become cationic before the polymer became associated with the salt, and the viscosity of the chitosan was essentially maintained. Apparently the viscous solution of the chitosan was not compatible with the solids of the sauce and there was an actual repulsion of the two entities. This repelling force was so great, that it resulted in the sedimentation of all the suspended solids from the majority of the liquid medium. This is indicated by the absence of any particles in the aqueous top layer of the higher concentrations of chitosan (Fig. 12). This would also explain the congealed phase of the hot sauce found in the preliminary investigation with Kytex. The solids of the suspension agglomerated in avoidance of the repelling viscous chitosan solution. This flocculation was not as great as with method No. 3 which used the high quality chitosan. Comparison of the viscosities of these two chitosan products are given in Fig. 9.

It appears that even a very high viscosity chitosan is not a suitable stabilizer for hot sauce. Superficially, chitosan's potential seems attractive since it is capable

of producing viscosities almost ten times greater than the xanthan gum. The composition of the hot sauce, however, either prevents the chitosan from forming viscous solutions or is incompatible with the viscous material once formed.

SUMMARY

A quality defect of Louisiana hot sauces is their tendency to separate in the bottle. This separation is prevented by the use of a hydrocolloid gum which thickens the sauce and stabilizes the separation. Chitosan is capable of forming viscous solutions in acetic acid. Since hot sauces are made with high quantities of vinegar, chitosan could thicken the sauce and prevent the separation.

High Grade Kytex, a commercially produced chitosan, was incorporated into hot sauces by first dissolving it in a one percent acetic acid solution and then combining it with the rest of the sauce's components. This method did not satisfactorily prevent the separation.

To improve the stabilizing effect of chitosan, a polymer capable of producing higher viscosities were produced. This was accomplished by using cold dilute HCl acid in the removal of the $CaCO_3$ in the shrimp shells in order to minimally degrade the chitin molecule. The chitin obtained was then deacetylated with forty percent NaOH at $100^{o}C$ for various times. It was found that deacetylation for four hours under these conditions produced the most viscous product. A one percent concentration of this material in 4.5% acetic acid produced a solution of 9600 cps.

Hot sauce was then produced with the addition of this higher viscosity chitosan. Three different methods of production were examined in order to determine which would be the most advantageous. Results showed the high salt content of the mash prevented chitosan from forming a viscous solution, if the chitosan was not first mixed with vinegar. When the viscous solution of chitosan and acetic acid was prepared first and then added to the rest of the constituents of the sauce, there was a promotion of the separation of the solids. This is the result of a repulsion of the two incompatible materials.

This investigation concludes that chitosan can not adequately prevent the separation of hot sauce.

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Approved:

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Date of Examination:

July 15, 1977