Recovery and Utilization of Chitin and Chitosan in Food Processing Waste Management

Waste products of the shellfish industry have applications as diet supplements, and in food processing waste management, beverage clarification, and the production of packaging films

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□ THE TREMENDOUS POTENTIAL of chitinous polymers for numerous applications has received limited recognition in the past. However, a significant amount of fundamental research on chitin and chitosan have been carried out since chitin was first described 180 years ago (Braconnot, 1811). Substantial research on chitin and chitosan has been performed especially during the last 20 years with four international conferences taking place and two significant publications appearing in 1989 (Skjåk-Braek et al., 1989; Pariser and Lombardi, 1989).

Chitin is a waste product of the seafood processing industry with an estimated 1.2×10^5 metric tons annually accessible on a worldwide basis (Table 1).

It is also estimated that fungi could provide 3.2×10^4 metric tons of chitin annually (Brine, 1984). Some values for chitin concentrations of mycelia of fungi are given in Table 2.

The two biopolymers chitin and chitosan (partially deacetylated chitin) offer a wide range of unique uses including clarification and purification of water and beverages; applications in pharmaceuticals and cosmetics; and agricultural, food, and biotechnology uses.

Total sales of chitin/chitosan are expected to reach almost 2 billion US dollars during the next ten years (Table 3). In the area of waste recovery and management two intriguing concepts for application and uses of chitinous polymers exist. One is the bioconversion of chitin/chitosan for the production of value added products and the other one is the utilization of the waste chitin/chitosan for the removal and recovery of other waste materials or valuable by-products. It is the aim of this paper to briefly review those two areas of chitin/chitosan utilization.

Recovery of Chitin and Chitosan

Main U.S. sources of shellfish that are processed into chitin and chitosan are Dungeness crabs (*Cancer*

Table 1—**Chitin from U.S. Crab** *and shrimp processing waste and global estimates of potential chitin sources (after Brine, 1984)*

Product	Dry waste (10^3 metric tons)		Chitin (10^3 metric tons)	
	Worldwide	U.S	Worldwide	U.S.
Shellfish	154		39	
Crab		17		6
Shrimp		78		39
Krill	801		56	
Clam/oyster	482		22	
Squid	21		1	
Total	1,458	95	118	45

Table 2—**Relative Amounts of Chitin** *in the mycelium of various fungi (after Ruiz-Herrera, 1978)*

Fungus	Chitin content (%)
Mucor rouxii	9.4
Aspergillus phoenicis	23.7
Aspergillus niger	42
Neurospora crassa	8.0–11.9
Penicillium chrysogenum	19.5–42
Trichoderma viridis	12–22
Saccharomycapsis gutulata	2.3
Blastomyces dermatitidis	13
Histoplasma capsulatum	25.8
Histoplasma farciminosum	40
Tremella mesenterica	3.7
Paracoccidioides brasiliensis	11

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magister), King crabs *(Paralithodes camschatica)* and the Pacific shrimp *(Pandalus borealis)*. The chitin and chitosan manufacturing process consists of removing proteins from ground shells by treatment with sodium hydroxide. Minerals such as calcium carbonate and calcium phosphate are removed by hydrochloric acid treatment. Deacetylation of chitin to chitosan is performed by treatment with sodium hydroxide to hydrolyze the N-acetyl-linkage, followed by rinsing, pH adjustment and drying.

Self-dissolving chitosan is prepared by blending chitosan powder with an organic acid (e.g. adipic acid) and purified chitosan can be processed by dissolving chitosan in an organic acid (e.g. acetic acid) followed by a filtration process (Sandford and Hutchings, 1987). A simplified flow chart of the chitin/chitosan manufacturing process is outlined in Figure 1.

Table 3—**Estimated Worldwide Sales** *of chitin and chitosan within 10 years (after Anonymous, 1989)*

Areas of application	Estimated sales (10^6 US$/year)
Agriculture	230
Cosmetics and toiletries	90
Food and beverages	110
Health care industry	1,250
Immobilization and cell culture	45
Product recovery and separation	50
Waste and water treatment	140
Total	1,915

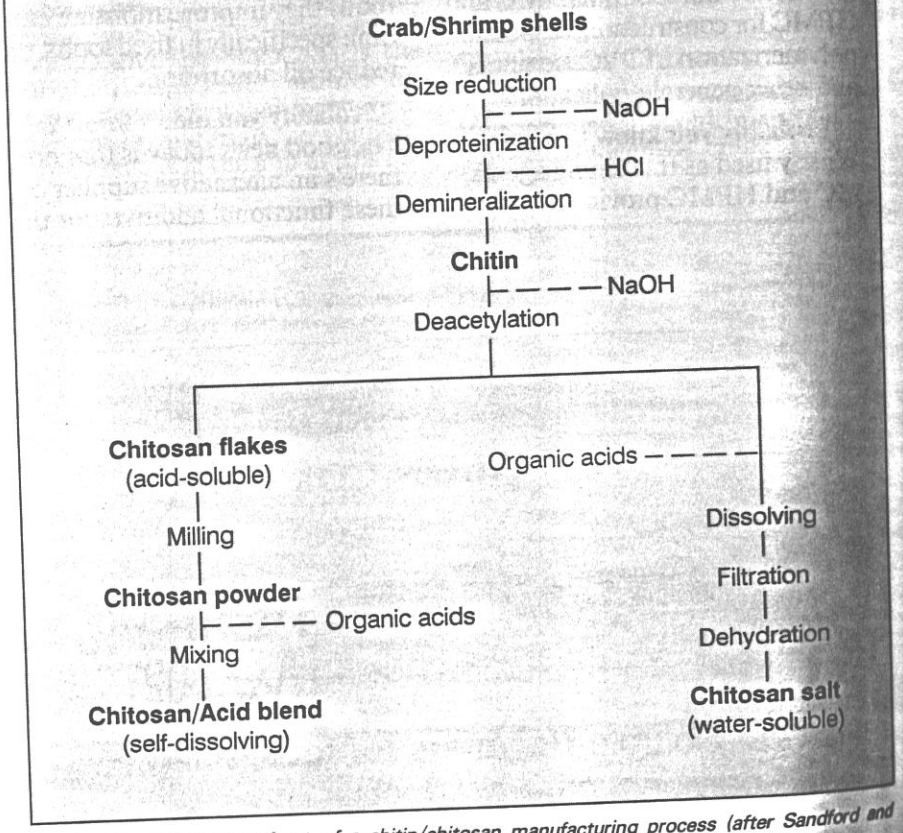

Fig. 1—**Simplified Flowchart** *of a chitin/chitosan manufacturing process (after Sandford and Hutchings, 1987)*

Conversion and Uses as Diet Supplements

Bioconversion of chitin to single-cell protein has been proposed by Carroad and Tom (1978) as a waste treatment alternative to the disposal of shellfish waste. The process concept consisted of size reduction of the chitin, protein and calcium carbonate containing shrimp waste. This was followed by protein removal by precipitation and by demineralization with acid. Some of the pretreated chitin was used for microbial chitinase production but the bulk of the chitin was hydrolized by chitinase. The yeast *Pichia kudriavzevii* was shown to grow well on the chitin hydrolysate at high temperature and low pH and to yield an acceptable amino acid distribution of the resulting protein fraction (Revah-Moiseev and Carroad, 1981). Based on these findings a process flow diagram and an economic analysis had been developed (Cosio et al., 1982). Screening of seventy-two strains of bacteria for ability to hydrolize chitin revealed 23 strains capable of hydrolyzing chitin (Cody et al., 1990).

Nutritional studies with broiler chicken have shown that microcrystalline chitin as diet supplements of up to 20% controlled diarrhea commonly occurring when whey is added to the chicken diet (Austin et al., 1981). According to the authors supplementation of chicken diets with chitin stimulated the growth of bifidobacteria that can synthesize lactase. Lactose has been the limiting factor for successful utilization of whey as chicken feed supplement (Spreen et al., 1984).

The effects of chitin, chitosan, and cellulose as diet supplements on the growth of cultured red sea bream, Japanese eel, and yellow tail have been investigated by Kono et al. (1990). The growth rate of all fishes fed with a 10% chitin supplement recorded the highest value indicating diet superiority. Feed efficiency in the red sea bream and Japanese eel which were fed with the 10% supplemented diet also recorded the highest values (Table 4).

Chitinase, chitosanase, and cellulose activity was measured in the stomach of the three types of fishes and only chitinase activity was de-

fected (Kono et al., 1987). The level of chitinase activity correlated with the growth rates of fish fed with the chitin supplemented diets and the authors concluded that chitin was digested and utilized by fishes with high chitinase activity in their digestive glands. This opens an interesting potential for fish farming since chitinase activity has also been detected in other fishes such as cod (Danulat, 1984). One also wonders what whales do with the 4 to 8 tons of krill (approx. 30 to 60 kg chitin) they consume daily.

The role of microbial and plant derived chitinases and their important role in the degradation of fungal cell walls should also be mentioned in this context (Oishi et al., 1989).

Hirano et al. (1990) recently provided data on chitin and chitosan digestibility by rabbits and hens (Table 5). No abnormal symptoms of chitosan supplement fed rabbits, broilers or hens were observed for up to 239 days with < 0.8 g chitosan/kg body weight/day and < 1.4 g chitosan/kg/d respectively. At concentrations of 3.6-4.2 g/kg/d physiological disorders could be observed in feeding experiments with hens for 189 days.

Chitosan supplements also decreased the concentrations of animal serum cholesterol (Table 5).

The nutritional significance and the digestion of chitinous waste materials as feedstuffs for cattle has been shown by Patton and Chandler (1975) and by Ortega and Church (1979). Watkins and Knorr (1983) evaluated the effects of dietary chitin supplements on growth and gut function of gerbils and Sugano et al. (1980) examined the effects of chitin and chitosan on growth of rats (Table 6). Up to 8.5% chitin addition and 10% chitosan addition no growth inhibition was reported. According to Arai et al. (1968) the LD50 dose of chitosan in laboratory animals is 16 g/kg body weight.

Table 4—**Diet Supplements. Effect of Chitin, Chitosan, and Cellulose as Diet Supplements** *in rearing of cultured fish during 30 days (after Kono et al., 1987)*

Fish type	Diet	Growth rate[a] %	Feed efficiency %
Red sea bream (N = 70)	Control	541	19.0
	5% chitin	550	19.2
	10% chitin	577	20.4
	20% chitin	548	19.1
Red sea bream (N = 40)	Control	253	12.6
	10% chitin	326	17.7
	10% chitosan	175	9.5
	10% cellulose	318	16.0
Japanese eel (N = 200)	Control	89	26.5
	10% chitin	99	29.8
	10% chitosan	24	7.3
	10% cellulose	61	18.2
Yellow tail (N = 50)	Control	177	20.0
	10% chitin	188	21.2
	10% chitosan	125	14.8
	10% cellulose	179	21.5

[a](Weight gain/initial body weight) × 100

Table 5—**Serum Cholesterol Values.** *Effects of chitin and chitosan supplements on their digestibility by rabbits and hens and on their serum cholesterol values (after Hirano et al., 1990)*

Supplement[a] (%)	Feeding period (d)	Digestibility (%)	
		Rabbits	Hens
Chitin (2)	25	35	—
	12	—	92
Chitosan (2)	5	41	—
	15	82	—
	39	83	—
Chitosan (5)	12	—	98
Chitosan (10)	12	—	67

		Total cholesterol serum (mg/dL)	
None	—	79 ± 4	—
Chitosan (1)	15	76 ± 0	—
Chitosan (2)	15	76 ± 0	—
Cholesterol (0.7)	39	850 ± 21	—
Chitosan (1) + cholesterol (0.7)	39	690 ± 13	—
Chitosan (2) + cholesterol (0.7)	39	300 ± 13	—
None	—	—	210 ± 10
Chitosan (5)	74	—	120 ± 10
Cholesterol (0.7)	81	—	370 ± 15
	189	—	670 ± 0
Chitosan (2) + cholesterol (0.7)	81	—	250 ± 15
	189	—	420 ± 0

[a]to basal diet

Biosorption of Heavy Metals, Dyes, and Pesticides

Higher awareness of the ecological and health effects of heavy metals and pesticides and their accumulation through the food chain has promoted a demand for purification of industrial waste waters prior to their discharge or their reuse. Conventional methods for the removal of metals from industrial waste solutions such as chemical precipitation or oxidation, filtration, electrochemical treatment, ion exchange, application of membrane technology and evaporation recovery, may be ineffective or expensive, espe-

cially when metals are available at low concentrations (Volesky, 1987). Naturally abundant biosorbents such as shellfish chitin/chitosan or chitin/chitosan containing microorganisms (Knorr and Klein, 1986) have been applied successfully. Muzzarelli and Tanfani (1982) presented data on the chelating ability of fungal waste mycelia which had been treated with boiling 40% sodium hydroxide to obtain insoluble chitosan-glucan complexes, for various metal ions. The effectiveness of crosslinked N-carboxymethyl chitosan in removing lead and cadmium from drinking waters has recently been demonstrated (Muzzarelli et al., 1989). Examples of adsorption of metal ions by chitosan from different sources are presented in Table 7.

It is also noteworthy that growth inhibition of *Chorella* cultures in the presence of copper and mercury ions was reduced in the presence of chitin and chitosan (Blair et al., 1982).

Adsorption of dyestuffs from processing effluents onto chitin has been reported by McKay et al. (1982) and dye binding properties of chitin have been investigated by Knorr (1983). In a pH range between 1 and 7 binding properties of chitin for FD&C Red No. 40 remained practically unchanged. Dye binding was between 0.7 and 0.8 mg dye per g chitin or chitosan. The kinetics of sorption of dyes on chitosan and the effects of temperature, particle size of chitosan, and pH of the dye solution have been examined by Venkatrao et al. (1986).

Purification of polychlorinated biphenyl (PCB) contaminated water with chitosan was shown to be apparently more effective than activated charcoal (Van Daele and Thomé, 1986; Thomé and Van Daele, 1986). It is interesting to note that these authors used the common barbel *(Barbus barbus)* as a biological test system for the efficiency of the process.

Protein Recovery

Data on the effectiveness of chitosan for the recovery of proteins from food processing wastes are presented in Table 8 and a comparison of various coagulating and flocculating agents is given in Table 9. These data indicate slightly higher reduction of turbidity and total suspended solids when chitosan was used at lower concentrations as compared to other commercially applied coagulating and flocculating agents.

Chitosan was also utilized as a coagulant for amino acids from crawfish processing waste water (No and Meyers, 1989) and flocculation of microalgae could be achieved at chitosan concentrations between 10 and 80 mg/L (Lubian, 1989).

Table 6—**Weight Gain.** *Effect of chitin and chitosan on weight gain, and liver weight of gerbils and rats (after Sugano et al., 1980, 1988; Watkins and Knorr, 1983)*

Dietary manipulation	Weight gain (g)	Liver weight (g/100 g body weight)
Control[a]	137 ± 7[A]	3.6 ± 0.1[A]
2% chitosan supplement[a]	137 ± 6[A]	3.6 ± 0.1[A,B]
5% chitosan supplement[a]	121 ± 11[A,B]	3.4 ± 0.1[A,B]
10% chitosan supplement[a]	96 ± 5[B]	3.2 ± 0.1[B]
Cellulose control[b]	144 ± 6	5.1 ± 0.1
25% chitosan[b]	135 ± 6 to 148 ± 7	4.3 ± 0.2 to 4.5 ± 0.2
Cellulose control[c]	218 ± 12	5.0 ± 0.1
4% chitosan	189 ± 5	3.0 ± 0.5
Cellulose control[d]	145 ± 5	4.7 ± 0.1
5% chitosan	104 ± 8 to 123 ± 7	3.6 ± 0.1 to 3.8 ± 0.2
	Weight (g)	
Control[e]	48.4 ± 3.4[A]	3.3 ± 0.3[A]
2.1 chitin[e]	49.5 ± 3.8[A]	2.8 ± 0.7[A]
4.2 chitin[e]	51.3 ± 2.8[A]	2.6 ± 0.3[A]
8.5 chitin[e]	51.8 ± 3.0[A]	2.8 ± 0.6[A]

[a]20 days experiment, age of rats at start of experiments 52 days (N = 6)
[A,B] values in the same column in each experiment sharing common superscript letters are insignificantly different at P > 0.05 to 0.01
[b]22 days experiment, initial age of rats 100 g, N = 12
[c]28 days experiment, initial weight of rats 100 g, N = 5
[d]21 days experiment, initial weight of rats 134 g, N = 3
[e]17 days experiment, gerbils, N = 4

Table 7—**Rate of Adsorption of Metal Ions** *by chitosan from different sources (after Ramachandran Nair and Madhavan, 1982)*

Metal ions	Chitosan source: Crab	Prawn	Squid	Squilla
	mg of metal adsorbed per g of chitosan[a]			
Fe^{3+}	17.6/23.4/23.4	11.7/15.7/23.4	17.6/20.5/23.4	14.6/17.6/29.3
Co^{2+}	4.1/ 4.7/ 5.9	5.3/ 7.1/ 7.1	4.7/ 4.7/ 7.4	4.7/ 4.7/ 4.7
Ni^{2+}	35.2/55.2/64.6	47.0/64.6/82.1	29.3/64.6/82.1	29.3/52.8/76.3
Hg^{2+}	241/281/321	311/331/341	321/346/366	351/381/411
Cu^{2+}	21.1/36.2/39.3	30.2/42.3/66.4	27.2/45.3/51.3	42.3/60.4/60.4

[a]First number = 30 min, second = 60 min, third = 120 min of treatment. 1 g of chitosan powder was added to 100 ml of 0.1 M solution of the metal ions.

Table 8—**Use of Chitosan in the Recovery of Proteins**

Protein source	Chitosan concentration (mg/L)	pH	Crude protein content of coagulated solids (% dry matter)	References
Cheese processing	2.5–15	6.0	78	Bough and Landes (1976); Wu et al. (1978)
Fruitcake processing	2	4.5	13-22	Bough (1976)
Meat processing	5-30	6.0-7.3	41	Bough (1976);
	15-40	7.4	32-51	Castellanos Perez et al. (1989)
Poultry processing	6-30	6.4-6.7	34-68	Bough (1975), 1976)
Crawfish processing	150	6.0	27	No and Meyers (1989)
Mussel processing	40	4.5	38	Holland and Shahbaz (1985)
Shrimp processing	60-360	5.5-6.0	[a]	Senstad and Almas (1986)

[a]65% protein recovery

Reduction of Microbial Counts

The antimicrobial potential of the polycationic chitosan has been discussed recently (Papineau et al., 1990; Popper and Knorr, 1990). Based on data on the effectiveness of chitosan for protein removal and recovery, a design for a combined process was attempted where microorganisms are removed by chitosan with concurrent protein removal/recovery.

The effects of various fining agents on turbidity and microbial counts of apple juice inoculated with *Lactobacillus plantarum* are demonstrated in Figure 3. These data suggest an effective reduction of turbidity as well as a reduction of microbial counts at ambient temperatures. This is of interest because of the current consumer demand for minimally processed foods. Even more dramatic effects on microbial populations could be observed when chitosan treatment was followed by a homogenization step (Popper and Knorr, 1990).

Affinity Purification

Chitosan, a natural polyligand rich in N-acetyl-D-glucosamine was—because of its specificity—effective in affinity precipitation of wheat germ agglutinin (Senstad and Mattiasson, 1989a). Since the polyligand is soluble at pH levels below 6.5 and precipitates at higher values it coprecipitated associated wheat germ agglutinin (WGA). The authors reported on overall yield of WGA of (70% and scale up of the procedure. Bloch and Burger (1974) used chitin as a ligand matrix and obtained an agglutinin that was homogenous with respect to polypeptide chain molecular weight, that had a blocked amino terminus and was free of proteolytic and β-N-acetyl-glucosaminidase activity.

Affinity-precipitation using chitosan as ligand carrier has been introduced by Senstad and Mattiasson (1989b). These authors indicated that this procedure presented a new and efficient way of utilizing affinity interactions in free solution and recovering the target molecule simply by precipitation. Trypsin could be precipitated at almost 100% using a soybean trypsin inhibitor-chitosan solution as the heterobiofunctional ligand.

Clarification of Beverages

Acid soluble crabshell chitosan and water soluble chitosan salt proved equally effective as fining agent for apple or carrot juices (Imeri and Knorr, 1988; Soto Peralta et al., 1989). The one step chitosan application was also found to be equal in effectiveness to the more cumbersome conventional silica sol/gelatin/bentonite treatment. As shown in Figure 2, 0.8 kg chitosan per m^3 of apple juice were sufficient to reach zero turbidity.

Biodegradable Packaging Films and Wildlife Protection

Environmental damage may occur through improper disposal of petrochemical based plastics such as six-pack straps. Bade and Wick (1988) reported that an estimated 30 percent of the fish in the world's oceans have pieces of plastics in their stomach that interfere with digestion. Based on the fact that chitin and chitosan have proven film forming properties (Averbach, 1978) and that chitosan degrading microorganisms are abundant (Fenton et al., 1978), Bade and Wick 1988) suggested the use of biodegradable

Table 9—**Space Effect of** *various coagulating and flocculating agents on the reduction of turbidity and total suspended solids of meat packing waste effluents (after Castellanos-Perez et al., 1989)*

Product	Concentration (mg/L)	Turbidity (final NTU)	Flocculation efficiency (%)	Total suspended[a] solids (mg/L)	Reduction of suspended solids (%)
Shrimp chitosan	20–40	2.0-4.9	94-98	4.5-11.1	95-98
Crab chitosan	15	4.4	96	14.0	95
Aspergillus niger chitosan-glucan complex	50-100	5.3-7.4	93-95	11.5-15.5	94-95
Cat-Floc[b]	40	4.4	96	17.7	93
$FeCl_3$	120	5.0	95	10.7	96
$Al_2(SO_4)_3$	150	5.2	95	11.7	95

[a]Initial concentration = 250 mg/L

[b]Commercial polymeric flocculating agent (Calgon Corp., Mexico City)

chitinous polymers for environmental protection and waste reduction. Mayer et al. (1989) recently demonstrated that underivatized chitosan had superior oxygen barrier properties but less tensile strength and flexibility compared with synthetic packaging films such as mylar and polypropylene.

When chitosan was crosslinked with epichlorohydrin the tensile strength of mylar and polypropylene was approached (Mayer et al., 1989). Also, an interesting concept was presented by Yang and Zall (1984) who fabricated alkali and acid resistant reverse osmosis membranes by acetylation of chitosan membranes.

What's Next?

The recovery of chitin and chitosan has become more sophisticated over the years resulting in highly purified or water soluble products. However, the recovery process itself could still be improved. For example, it seems imaginable that proteases are being utilized for protein removal and it is surprising that chitosan is not being utilized for the recovery of crab/shrimp proteins or amino acids during the processing of chitin. In addition, chitin and chitosan could have a significant impact on the seafood industry as dietary supplement in fish farming and waste removal including proteins, heavy metals, and pesticides.

The removal of proteins, heavy metals, dyes, and pesticides from liquid food systems by chitin or chitosan is of general importance for the food and feed industry. Also, affinity purification, clarification of beverages, reduction of microbial counts are important features of chitosan applications for waste management and reduction.

Finally, due to the biodegradability of the chitinous polymers, chitin and chitosan can provide essential contribution to the reduction of packaging waste and to wildlife protection.

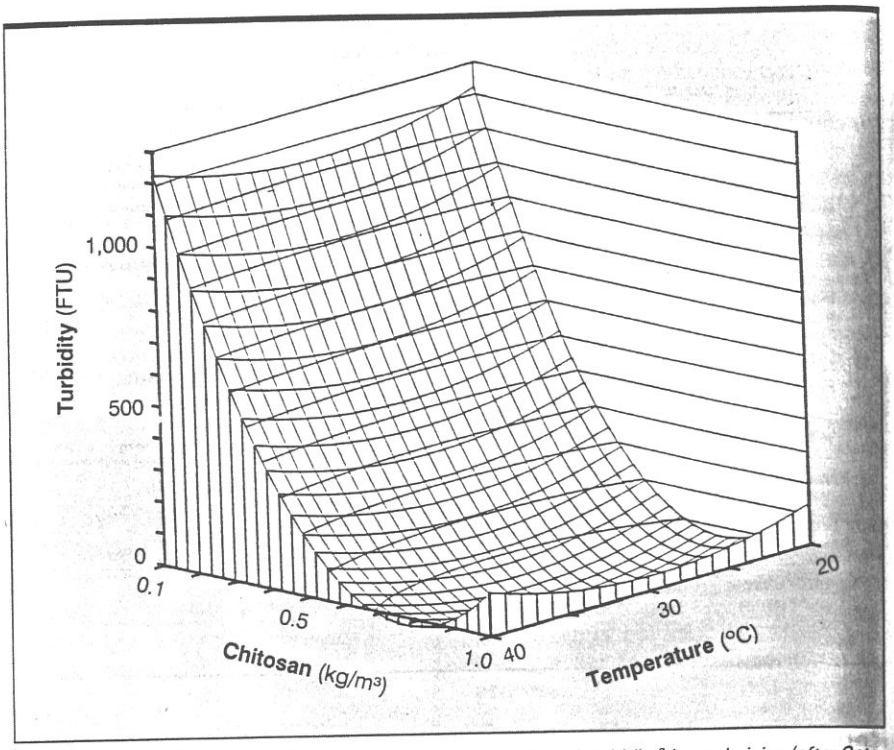

Fig. 2—**Effect of Water Soluble Chitosan** *on the reduction of turbidity[a] in apple juice (after Soto-Peralta et al., 1989*
[a]formazin turbidity units

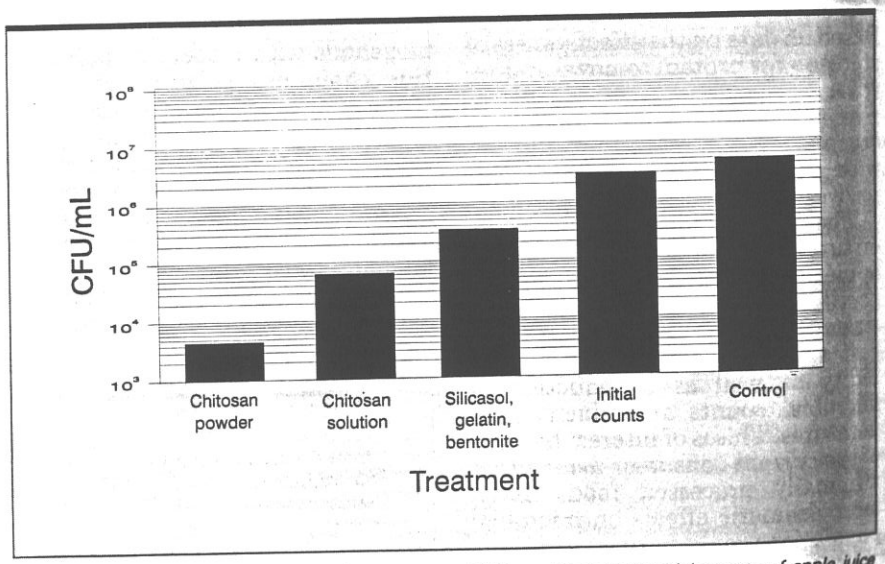

Fig. 3—**Effects of Various Fining Agents** *on turbidity and total microbial counts of apple juice inoculated with* Lactobacillus plantarum *(after Boguslawski et al., 1990). Chitosan powder (1g/L), chitosan solution (2g/L), silicasol (3g/L)/gelatin (0.1g/L)/bentonite (0.5g/L); control = fining treatment without polymer addition*

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Publication Announcement

Title **Arsenic sorption by chitosan and chitin deacetylase production by *Mucor rouxii***

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Summary In Japan, chitosan from shellfish is used in million-kilogram quantities as a sorbent in the clarification of municipal and industrial wastes. Chitosan is produced from chitin by treating shellfish with the enzyme chitin deacetylase. Because processing shellfish shells in this way is expensive and results in a product of variable quality, the authors studied propagating and harvesting a chitosan-rich fungus for use as an alternative sorbent.

In addition to demonstrating that chitosan effectively absorbs arsenate, the authors conclude that the hyphae of the fungus *Mucor rouxii* can be useful for biorecovery of anionic metals. They also conclude that chitosan plays a regulatory role in apical growth and germ tube emergence of this fungus. They encourage continuing studies of chitosan synthesis and function in microorganisms, with the goal of commercially producing chitosan and chitosan-rich hyphae using fermentation methods.

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