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Article *in* Trends in Food Science & Technology · February 1999

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Trends in Food Science & Technology 10 (1999) 37–51

TRENDS IN FOOD SCIENCE & TECHNOLOGY

Review

Food applications of chitin and chitosans

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Chitin is the second most abundant natural biopolymer after cellulose. The chemical structure of chitin is similar to that of cellulose with 2-acetamido-2-deoxy-β-D-glucose (NAG) monomers attached via β(1→4) linkages. Chitosan is the deacetylated (to varying degrees) form of chitin, which, unlike chitin, is soluble in acidic solutions. Application of chitinous products in foods and pharmaceuticals as well as processing aids has received considerable attention in recent years as exotic synthetic compounds are losing their appeal. This review summarizes some of the important developments related to food applications of chitin, chitosan and their derivatives. © 1999 Elsevier Science Ltd. All rights reserved.

The name 'chitin' is derived from the Greek word 'chiton', meaning a coat of mail [1], and was apparently first used by Bradconnot in 1811 [2]. It is the second most abundant biopolymer on earth after cellulose and is a β(1→4)-linked glycan, but is composed of 2-acetamido-2-deoxy-β-D-glucose (*N*-acetylglucosamine), one of the most abundant polysaccharides [1] named polyβ(1→4)-2-acetamido-2-deoxy-D-glucose. Chitosan is the name used for low acetyl substituted forms of chitin and is composed primarily of glucosamine, 2-amino-2-deoxy-β-D-glucose, known as (1→4)-2-amino-2-deoxy-(D-glucose (Fig. 1). Chitosan has three types of reactive functional groups, an amino group as well as both primary and secondary hydroxyl groups at the C-2, C-3 and C-6 positions, respectively [3]. Chemical modifications of these groups have provided numerous useful materials in different fields of application [4] (Fig. 2).

Chitin is the major structural component of the exoskeleton of invertebrates and the cell walls of fungi [1, 5–8]. Since the biodegradation of chitin is very slow in crustacean shell waste, accumulation of large quantities of discards from processing of crustaceans has become a major concern in the seafood processing industry [9]. Out of the total solid waste landing in the USA, 50–90% is from shellfish processing discards [10], the total global annual estimates of it is around 5.118×10^6 metric tons [5]. Out of the different species of crustaceans, shrimp and crab shell wastes have been widely used for the isolation of chitin [11, 12]. Disposal of shellfish processing discards has, thus, been a challenge for most of the shellfish-producing countries. Therefore, production of value-added products such as chitin, chitosan and their derivatives and application of products in different fields is of utmost interest. Meyers and Chen [13] as well as Shahidi and Synowiecki [9] have reported the economical acceptability of this industry with further extraction of pigments, proteins and carotenoproteins from processing discards of shrimp and crab.

Chitin and its deacetylated form, chitosan, have been of interest in the past few decades due to their potential broad range of industrial applications [7, 14]. However, only limited attention has been paid to food application of these versatile biopolymers. Conversion of processing discards into valuable by-products and alternative specialty materials has been identified as a timely challenge for food research and development associated with numerous applications of chitinous polymers. In that sense, these biopolymers offer a wide range of unique applications including bioconversion for the production of value-added food products [9, 15, 16], preservation of foods from microbial deterioration [17–21], formation of biodegradable films [22–27], recovery of waste material from food processing discards [28–35], purification of water [36–39] and clarification and deacidification of fruit juices [40–44] (Table 1).

Antimicrobial activity of chitin, chitosan and their derivatives

The growing consumer demand for foods without chemical preservatives has focused efforts in the discovery of

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PII: S0924-2244(99)00017-5

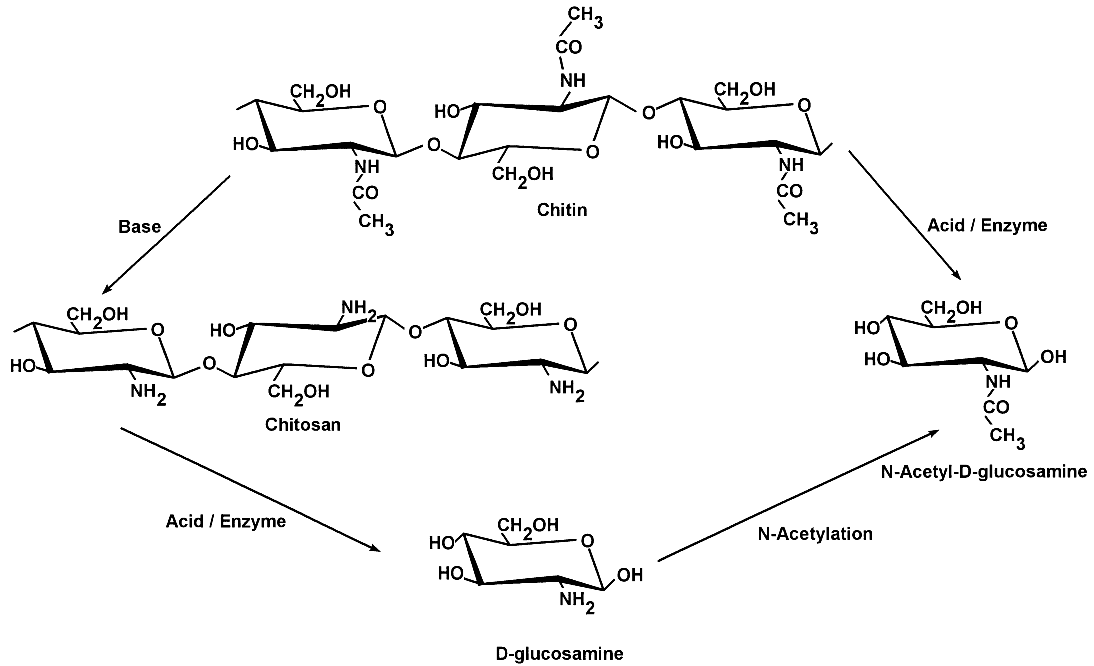

Fig. 1. Preparation of Chitin derivatives from chitin.

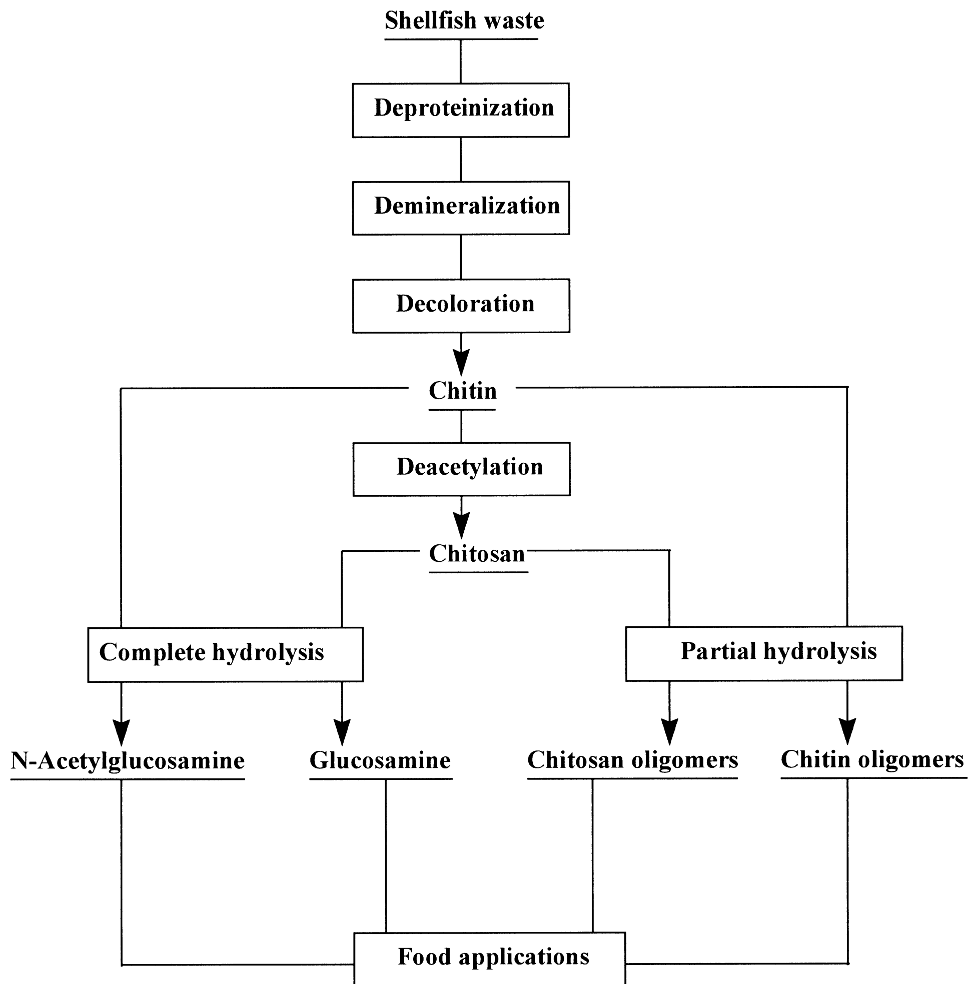

Fig. 2. Simplified flowsheet for preparation of chitin, chitosan, their oligomers and monomers from shellfish waste.

new natural antimicrobials [45]. In this context, the unusual antimicrobial activity of chitin, chitosan and their derivatives against different groups of microorganisms, such as bacteria, yeast and fungi has received considerable attention in recent years [46].

Because of the positive charge on the C-2 of the glucosamine monomer at below pH 6, chitosan is more soluble and has a better antimicrobial activity than chitin [21]. The exact mechanism of the antimicrobial action of chitin, chitosan and their derivatives is still unknown, but different mechanisms have been proposed. Interaction between positively charged chitosan molecules and negatively charged microbial cell membranes leads to the leakage of proteinaceous and other intracellular constituents [17, 19-21, 47]. Chitosan also acts as a chelating agent that selectively binds trace metals and thereby inhibits the production of toxins and microbial growth [48]. It also activates several defense processes in the host tissue [18], acts as a water binding agent and inhibits various enzymes [47]. Binding of chitosan with DNA and inhibition of mRNA synthesis occurs via chitosan penetrating the nuclei of the microorganisms and interfering with the synthesis of mRNA and proteins [19, 49].

Antimicrobial activity of chitin, chitosan and their derivatives against bacteria

Wang [45] observed that a much higher concentration of chitosan (1–1.5%) is required for complete inactivation of *Staphylococcus aureus* after two days of incubation at pH 5.5 or 6.5 in the medium. Furthermore, Chang *et al.* [50] found that chitosan concentrations of $\geq$0.005 were sufficient to elicit complete inactivation of *S. aureus*. This was in accordance with the findings of Darmadji and Izumimoto [51] on the effect of chitosan in meat preservation. Simpson *et al.* [52] studied the antimicrobial effect of different cultures of bacteria on raw shrimp, with different concentrations of chitosan and observed variations in their degree of susceptibility to chitosan. According to their findings, *Bacillus cereus* required chitosan concentrations of (0.02% for bactericidal effect, while *Escherichia coli* and *Proteus vulgaris* showed minimal growth at 0.005%, and complete inhibition at $\geq$0.0075%. Chang *et al.* [50] also reported inhibition of *B. cereus* by chitosan. However, much lower concentrations (0.005%) were required, perhaps due to the low molecular weight (35 kD) of chitosan used in their experiment. Numerous studies have also shown the effect of chitosan on *E. coli* inhibition. Wang [45] observed complete inactivation after a 2-day incubation period with concentrations of 0.5 or 1%, at pH 5.5. He also reported that complete inactivation could be reached even after first day, if the chitosan concentration is more than 1% in the broth. Meanwhile, Darmadji and Izumimoto [51] reported that higher concentrations (0.1%) were required to inhibit *E.coli* growth and Simpson *et al.* [52] found that only 0.0075% chitosan was needed to inhibit the growth of *E.coli*. These variations were suggested to be due to the existing differences in the degree of acetylation of chitosan; chitosan with a degree of acetylation of 7.5% was more effective than chitosan with a degree of acetylation of 15%.

Sudharshan *et al.* [19] studied the antimicrobial effect of water-soluble chitosans such as chitosan lactate, chitosan hydroglutamate and chitosan derived from *Absidia coerulea* fungi, on different bacterial cultures. They observed that chitosan glutamate and chitosan lactate were also bactericidal against both gram-positive and gram-negative bacteria in the range of one to five log cycle reduction within one hour. In that same study these authors reported that chitosan was no longer bactericidal at pH 7 due to two major reasons, namely presence of a significant proportion of uncharged amino groups and poor solubility of chitosan. These results are in agreement with findings of Papineau *et al.* [17] in a similar study where a concentration of 0.2 mg/mL chitosan lactate appeared most effective against *E.coli* with a corresponding population drop of 2 and 4 log cycles within 2 min and 1 h exposure, respectively. These authors observed that chitosan glutamate was also effective against yeast cultures such as *Saccharomyces cerevisiae* and *Rhodotorula glutensis* and inactivation was rapid and complete within 17 min when exposed to 1 mg/mL chitosan lactate. However, in contrast to the findings of Sudharshan *et al.* [19], Papineau *et al.* [17] observed that chitosan hydroglutamate was a more effective antagonist than chitosan lactate. Results further suggested that chitosan acted mainly on the outer surface of the bacteria. At a lower concentration, the polycationic chitosan did probably bind to the negatively charged bacterial surface to cause agglutination, while at higher concentrations the larger number of positive charges may have imparted a net positive charge to the bacterial surfaces to keep them in suspension.

In another study, Chen *et al.* [21] reported the antibacterial effects of 69% deacetylated shrimp chitosan, 0.63% sulphonated chitosan (SC1), 13.03% sulphonated chitosan (SC2) and sulphobenzoyl chitosan on oyster preservation. They observed that, except in the case of *B.cereus*, bacterial growth was effectively inhibited by at least one of the above four compounds tested at 200 ppm. Even though the sulphonation increased the solubility of chitosan, totally different antibacterial capabilities were observed for SC1 and SC2. For most of the bacterial cultures SC1 had a very pronounced minimal inhibitory concentration (MIC) effect even at 200 ppm level, with SC2 exhibiting no antibacterial effect at concentrations below 2000 ppm. Chen *et al.* [21] suggested that since SC2 has more sulphonyl groups, it carries a higher negative charge than SC1, thus there would be a greater repulsive force between negatively charged SC2 molecules and bacterial cell walls (Table 2).

Table 1. Food applications of chitin, chitosan and their derivatives in the food industry

Area of application	Examples
Antimicrobial agent	Bactericidal Fungicidal Measure of mold contamination in agricultural commodities
Edible film industry	Controlled moisture transfer between food and surrounding environment Controlled release of antimicrobial substances Controlled release of antioxidants Controlled release of nutrients, flavours and drugs Reduction of oxygen partial pressure Controlled rate of respiration Temperature control Controlled enzymatic browning in fruits Reverse osmosis membranes
Additive	Clarification and deacidification of fruits and beverages Natural flavour extender Texture controlling agent Emulsifying agent Food mimetic Thickening and stabilizing agent Colour stabilization
Nutritional quality	Dietary fibre Hypocholesterolemic effect Livestock and fish feed additive Reduction of lipid absorption Production of single cell protein Antigastritis agent Infant feed ingredient
Recovery of solid materials from food processing wastes	Affinity flocculation Fractionation of agar
Purification of water	Recovery of metal ions, pesticides, phenols and PCB's Removal of dyes
Other applications	Enzyme immobilization Encapsulation of nutraceuticals Chromatography Analytical reagents

Antimicrobial activity of chitin, chitosan and their derivatives against fungi

Use of bioactive substances such as chitosan to control post-harvest fungal disease has attracted much attention due to imminent problems associated with chemical agents, which include development of public resistance to fungicide-treated produce, and increasing the number of fungicide tolerant post-harvest pathogens and a number of fungicides that are still under observation [18]. Chitosan reduces the *in vitro* growth of numerous fungi with the exception Zygomycetes, i.e the fungi containing chitosan as a major component of its cell walls [53]. In addition to the formation of gas permeable films, chitosan has a dual function, that is direct interference of fungal growth and activation of several defense processes [54]. These defense mechanisms include accumulation of chitinases, synthesis of proteinase inhibitors, and lignification and induction of callous synthesis [55].

The antifungal effect of chitosan on *in vitro* growth of common post-harvest fungal pathogens in strawberry fruits was studied by El Ghaouth *et al.* [18]. According to that study, chitosan (with 7.2% NH_2) reduced markedly the radial growth of *Botrytis cinerea* and *Rhizopus stolonifer*, with a greater effect at higher concentrations. These authors further confirmed the importance of a large number of alternating positively charged groups along the length of the polymer chain because low antifungal activity was observed with *N,O*-carboxymethylchitosan compared with that of chitosan itself [18]. In an *in vivo* study, El Ghaouth *et al.* [55] reported signs of infection in chitosan-coated fruits after 5 days of storage at 13°C compared with 1 day for the control treatment. After 14 days of storage, chitosan coating at 15 mg/mL reduced decay of strawberries caused by the same fungi by more than 60%, and also observed that coated fruits ripened normally and did not show any apparent sign of phytotoxicity. In another study, Fang *et al.* [20] reported the preservative effect of chitosan on low-sugar candied kumquat (fruit). The growth of *Aspergillus niger* was inhibited by the addition of chitosan (0.1–5 mg/mL) to the medium (pH 5.4), whereas at less than 2 mg/mL chitosan was not effective in inhibiting mold growth and aflatoxin production by *Aspergillus parasiticus*. In a similar study, Cuero *et al.* [48] observed that *N*-carboxymethylchitosan reduced aflatoxin production in *A. flavus* and *A. parasiticus* by more than 90% while fungal growth was reduced to less than half. Savage and Savage [56] also reported that apples coated with chitosan reduced the incidence of molds occuring on the apples over a period of 12 weeks. A study carried out on chitosan coating for the inhibition of Sclerotinia rot of carrot showed that the incidence of rotting was significantly reduced (from 88 to 28%) by coating carrot roots with 2 or 4% chitosan [57]. Chitosan also induced a plant-defense enzyme, chitinase, in plant tissues, which degrades fungal cell walls [58], and induced the accumulation of the antifungal phytoalexin pisatin in pea pods [59, 60]. These results suggest that coating fruits and vegetables with chitosan or its derivatives may have some positive advantages for long term storage of these foods.

Chitin as a measure of mold contamination of agricultural commodities and food products

It is important to enumerate and identify molds that occur in food commodities for both quality assurance and control operations in food processing plants [61].

Table 2. Minimal inhibitory concentrations (MIC, ppm) of chitosan and derivatives for different bacterial cultures[a]

Bacterial culture	DD69	SC1	SC2	SBC
Gram positive				
Staphylococcus aureus	100	100	> 2000	200
Listeria monocytogenes	100	100	> 2000	100
Bacillus cereus	1000	500	NT	> 2000
Gram negative				
Escherichia coli	100	100	NT	100
Vibrio parahaemolyticus	100	100	> 2000	100
Pseudomonas aeruginosa	200	200	> 2000	2000
Shigella dysenteriae	200	100	> 2000	100
Vibrio cholerae	200	> 2000	> 2000	2000
Aeromonas hydrophila YMI	500	200	> 2000	200
Aeromonas hydrophila CCRC 13881	2000	200	> 2000	500
Salmonella typhimurium	> 2000	200	> 2000	2000

[a]Symbols are: DD69—69% deacetylated chitosan; SCl—Sulphonated chitosan—0.63% S; SC2—Sulphonated chitosan—13.03% S; SBC–Sulphobenzoyl chitosan; NT—Not tested Data adapted from Chen *et al.* [2]

Quantitative determination of the number of fungi present on the surface of stored products does not include non-viable mycelium [62] since the Howard mold count method requires special training and experience to detect fungi; also, results are very variable due to milling and standardization of the food [61]. Chemical determination of chitin, a constituent of fungal cell walls, has an advantage in that it will reflect total mycelium based on chitin content [62, 63]. Bishop *et al*. [63] used chitin to further evaluate the detection of mold in tomato products, ketchup, paste and puree. Variations were observed in chitin content among different fungal species, depending upon cultural age, growth conditions and values ranged from 5.7 to 43 μg of glucosamine per mg dry weight. Bishop *et al*. [63] also concluded that insect contamination did not change the glucosamine level significantly until extremely high levels of contamination.

Chitin and chitosan in the edible film industry

The use of edible films and coatings to extend shelf life and improve the quality of fresh, frozen and fabricated foods has been examined during the past few years [64, 65] due to their ecofriendly and biodegradable nature [26, 27]. These outer layers/films can provide supplementary and sometimes essential means of controlling physiological, morphological and physicochemical changes in food products [27]. High density polyethylene film, a common packaging material used to protect foods [66], has disadvantages like fermentation due to the depletion of oxygen [67] and condensation of water due to fluctuation of storage temperature, which promotes fungal growth [68]. There are many mechanisms involved in extending shelf life of food by coating films. These include controlled moisture transfer between food and surrounding environment, controlled release of chemical agents like antimicrobial substances, antioxidants, reduction of oxygen partial pressure in the package that results in a decreased rate of metabolism, controlled rate of respiration, high impermeability to certain substances like fats and oils, temperature control, structural reinforcement of food and coat flavour compounds and leavening agents in the form of microcapsules [64, 65].

Due to their film-forming properties, chitin [69] and chitosan [70] have been successfully used as food wraps. The use of *N,O*-carboxymethylchitin films to preserve fruits over long periods has been approved in both Canada and the USA [71]. Due to its ability to form semipermeable film, chitosan coating can be expected to modify the internal atmosphere as well as decrease the transpiration loss [72] and delay the ripening of fruits [67].

Rigid chitosan films can be formed using crosslinking agents such as glutaraldehyde [23], divalent metal ions, polyelectrolytes [73], or even anionic polysaccharides [26]. The preparation of chitosan and chitosan laminated films with other polysaccharides has been reported by various authors; these include chitosan films [24, 25, 27], chitosan/pectin laminated films [26] and chitosan/methylcellulose films [74]. Several approaches have been used to form these edible films or coatings, including simple coacervation, where a single hydrocolloid is transfering from aqueous suspension or caused to change its phase by evaporation of the solvent. In addition, complex coacervation, where two solutions of oppositely ionized hydrocolloids are united, causing interaction and precipitation of the polymer complex as well as simple cooling of a warm hydrocolloid suspension to bring about a sol–gel transformation has been practiced [64].

Chitosan films are tough, longlasting, flexible and very difficult to tear. Most of these mechanical properties are comparable to many medium-strength commercial polymers [24]. Kittur *et al*. [27] reported that chitosan films have moderate water permeability values and could be used to increase the storage life of fresh

produce and foodstuffs with higher water activity values. However, Wong *et al.* [22] and Butler *et al.* [24] observed extremely good barriers to permeation of oxygen, while exhibiting relatively low vapour barrier characteristics. By incorporating fatty materials, hydrophobicity can be increased thereby producing composite films resistant to water transmission [22].

Effect of chitosan coating on storability and quality of fresh fruits

Extension of the storage life and better control of decay of peaches, Japanese pears and kiwifruits by application of chitosan film has been documented [75]. Similarly, cucumbers, and bell peppers [76], strawberries [72], and tomatoes [67] could be stored for long periods after coating with chitosan. These results may be attributed to decreased respiration rates, inhibition of fungal development and delaying of ripening due to the reduction of ethylene and carbon dioxide evolution [18,75,76].

Antimicrobial properties of chitosan and chitosan-laminated films

Chitosan and chitosan-laminated films containing antimicrobial agents provide a type of active package such that the preservatives released from the film deposit on the food surface and inhibit the microbial growth [65, 74]. Torres *et al.* [77] evaluated the sorbate-loaded edible barrier for mold inhibition on food surfaces, and Field *et al.* [78] advocated the use of glucose oxidase/glucose as a dip for extension of shelf life of fish. The presence of preservatives in chitosan films reduces the intermolecular electrostatic repulsion in the chitosan molecules and facilitates formation of intramolecular hydrogen bonds [79]. Chen *et al.* [74] have observed that the packaging film prepared from methylcellulose, chitosan and preservatives possesses antimicrobial activity.

Control of enzymatic browning in fruits by chitosan films

Mechanical injury during post-harvest handling and processing causes browning of fruits and vegetables with greater loss of quality and value [80, 81]. Phenolic compounds, together with the activity of polyphenol oxidase (PPO), are responsible for this phenomenon and will affect the colour, taste and nutritional value of fruits and vegetables [82]. Dark-coloured pigments, which are polymerized products of *o*-quinones, are formed due to polyphenol oxidase activity [83, 84]. In recent years, concern over the adverse health effects of sulphite, the most effective browning inhibitor, has stimulated a search for alternate antibrowning compounds [85]. The effect of application of a chitosan coated film on enzymatic browning of litchi (*Litch chinensis* Sonn.) fruit was studied by Zhang and Quantick [86] who reported that chitosan film coating delayed changes of contents of anthocyanins, flavonoids and total phenolics. It also delayed the increase in polyphenol oxidase activity and partially inhibited the increase in peroxidase activity. These authors further reported that application of chitosan may form a layer of film on the outer pericarp surface, thus resulting in less browning.

Application of chitosan for clarification and deacidification of fruit juices (fining agent)

Processing of clarified fruit juices commonly involves the use of clarifying agents, including gelatin, bentonite, silica sol, tannins [87], potassium caseinate and polyvinyl pyrrolidone [44]. Chitosan salts, which carry a strong positive charge, have been shown to be effective as dehazing agents; they may also be used to control acidity in fruit juices [40]. Chitosan is a good clarifying agent for grapefruit juice either with or without pectinase treatment [42] and highly effective fining agent for apple juice, which can afford zero turbidity products with 0.8 kg/m^3 of chitosan [41]. In a similar study, Spagna *et al.* [44] observed that chitosan has a good affinity for polyphenolic compounds such as catechins, proanthocyanidins, cinnamic acid and their derivatives that can change the initial straw-yellow colour of white wines into deep golden-yellow colour due to their oxidative products. By adding chitosan to grapefruit juice at a concentration of 0.015 g/mL, total acid content was reduced by about 52.6% due to decreasing the amount of citric acid, tartaric acid, L-malic acid, oxalic acid and ascorbic acid, by 56.6, 41.2, 38.8, 36.8 and 6.5%, respectively [43].

Recovery of solid materials from food processing wastes and water

Adjustment to comply with water quality regulations is one of the major challenges of the food industry in recent years [35]. Organically rich effluents from food processing plants are characterized by high chemical oxygen demand (COD) or biochemical oxygen demand (BOD) and total suspended solids [88]. Recovery of suspended solids by coagulation and settling may also be cost effective for reducing surcharges [29, 30, 34] in terms of their potential utilization [88].

The concept of using affinity interactions, mainly for isolation of waste materials from food processing waste, is very attractive because a high resolution technique is used initially in the purification scheme, thereby making it possible to reduce the volume of sample handled in later steps [89]. During the past decade increasing attention has been paid to the polyelectrolytic coagulants of natural origin.

Chitin and chitosan as coagulation and flocculation substances in food processing waste water

Chitosan, with its partial positive charge, can effectively function as a polycationic coagulant in wastewater treatment [90]. Chitosan as a coagulating agent for

waste treatment systems [28] is particularly effective in removing proteins from wastes; the coagulated by-products could serve as a source of protein in animal feed [29]. Chitosan reduced the suspended solids of various food processing wastes to different contents (Table 3). There are two stages that result in destabilization of a colloidal system; coagulation and flocculation. The former is the process where the forces holding the particles in suspension are neutralized, whereas flocculation is the process in which destabilized suspended particles are brought together to form larger aggregates [31, 35]. The mechanism of flocculating lipids and proteins from food processing waste is due to the pKa of the amino group of glucosamine residue which is about 6.3 [91], hence chitosan is polycationic at acidic pH values [33, 92]. Thus, in contrast to chitin, chitosan is soluble at pH below 6.3 and precipitates at higher pH values [32].

Application of chitosan for recovery of protein and fat from cheese whey

Fernandez and Fox [92] reported the use of chitosan to remove proteins and peptides from cheese whey. Urea-PAGE (polyacrylamide gel electroplorosis) showed that chitosan gave good fractionation of water-soluble extract at pH 2, 3 and 4. At pH 5, 6 and 7, most of the nitrogen of the water-soluble extract remained soluble in 0.02% chitosan. However, when the pH was reduced stepwise, the best fractionation was obtained at pH 4.0. The addition of 0.01–0.016% chitosan to cheddar cheese whey at pH 4.5 almost completely removed the milkfat globule membrane fragments prior to ultrafiltration [33]. Since the pKa of the amino group of glucosamine residues is about 6.3, chitosan is extremely positively charged at pH 4.5. This facilitates electrostatic interaction between chitosan and the negatively charged milkfat globule membrane fragments. This method could be used as an industrially feasible process to remove lipids from cheese whey [33]. Coagulation of cheese whey with chitosan demonstrated that the optimum percentage of chitosan suspended solids was 2–2.5% at pH 6. This corresponded to chitosan concentrations of 49–62 mg/L for whey containing an average concentration of 2270 mg/L of suspended solids (SS). A 90% reduction in SS was achieved by this treatment [30]. Greater reductions in turbidity of cheese whey were observed as chitosan concentration was increased [93]. The influence of different factors such as ionic strength, pH, size of the drops in the emulsion, relative concentration of oil and emulsifier and type of emulsifier on the dose of chitosan necessary to obtain flocculation of the model food oil/water emulsion was observed by Pinotti *et al.* [35]. These authors reported that the increase in NaCl concentration reduces the dose of chitosan necessary to produce destabilization and flocculation. The longer the surfactant chain length, the greater the tendency toward polyelectrolyte association, therefore the greater was the chitosan dose to reach zero charge.

Application of chitin and chitosan for purification of water

Better awareness of the ecological and health problems associated with heavy metals and pesticides and their accumulation through the food chain has prompted the demand for purification of industrial waste waters prior to their discharge or use [36, 94]. Conventional methods for the removal of metals from industrial waste water, may be ineffective or expensive, especially when metals are available at low concentrations [39, 95].

Chelation ion exchange is a technique which can be used to recover metal ions from waste water. Commercially available and environmentally safe biopolymers are capable of lowering transition metal ion concentrations to parts per billion levels. Such biopolymers possess a number of different functional groups, such as

Table 3. Effect of chitosan on reduction of solid materials from food processing wastes

Type of waste	Chitosan amount (mg/L discharge)	Reduction of suspended solids (%)	Reference
Meat processing[a]	30	89	[29]
Shrimp processing[b]	10	98	[29]
Crawfish processing	150	97	[88]
Cheese whey	2.5–15	82–97	[30]
	10–16	74	[33]
Poultry processing[c]	30	88	[11]
Egg processing[d]	100–200	70–90	[29]
Wheat germ agglutinin	20	70	[32]
Vegetable processing	10	84–90	[28]
Fruitcake processing	2	94	[29]

[a]Packing waste water.
[b]With anionic polymer.
[c]Chiller discharge.
[d]With cationic polymer.

hydroxyl and amino moieties, that can be used to increase the efficiency of metal ion uptake [39]. Chitosan can be utilized as a tool for the purification of waste water because of its high sorption capacity [36]. The capacity of chitin and chitosan to form complexes with metal ions has been exploited in Japan for water purification [87]. The NH_2 group of chitosan is of interest due to its ability to form coordinate covalent bonds with metal ions. Chitosan powder and dried films have more potential use in metal ion complexing because it will release most of its free amino groups above the pKa of the NH_2 group of chitosan [96].

The use of commercially available chitosan for potable water purification has been approved by the United States Environmental Protection Agency (USEPA) up to a maximum level of 10 mg/L [5]. The effectiveness of cross-linked *N*-carboxymethylchitosan in removing lead and cadmium from drinking water has been demonstrated by Muzzarelli *et al.* [38]. A study of the metal binding capacity of chitosan has shown that it has a high binding capacity with metals such as copper and vanadium [37]. Deans and Dixon [39] have reported that unfunctionalized chitosan is effective in removing Cu^{2+}, but not Pb^{2+}. However, for copper at 10 and 100 ppm, the best adsorbents were found to be carboxymethylchitosan and the ion exchange resin, respectively. The ability of chitosan to remove polychlorinated biphenyls (PCB) from contaminated stream water was tested by Thome and Daele [97] who demonstrated that chitosan was highly efficient and more effective than activated charcoal for purification of PCB contaminated water.

Antioxidative properties of chitosan and chitosan derivatives on muscle foods

Muscle food products are highly susceptible to off-flavour and rancidity development caused by oxidation of their highly unsaturated lipids. Warmed-over flavour in cooked poultry and uncured meat is developed upon storage and results in the deterioration of desirable meaty flavour. Effectiveness of chitosan treatment on oxidative stability of beef was studied by Darmadji and Izumimoto [51] who observed that addition of chitosan at 1% resulted in a decrease of 70% in the 2-thiobarbituric acid (TBA) values of meat after 3 days of storage at 4°C. The effect of *N*-carboxymethylchitosan to prevent the warmed-over flavour (WOF) in uncured meat was studied by St. Angelo and Vercellotti [98]. These authors reported that *N*-carboxymethylchitosan was effective in controlling WOF over a wide range of temperature. Use of 5000 ppm *N*-carboxymethylchitosan in ground beef resulted in a 93% inhibition of TBA and 99% reduction in the hexanal content in the products. However, Shahidi [99] reported that *N,O*-carboxymethylchitosan (NOCC) and its lactate, acetate and pyrrolidine carboxylate salts were effective in controlling the oxidation and flavour deterioration of cooked meat over a nine day storage at refrigerated temperatures. The mean inhibitory effect of NOCC and its aformentioned derivatives at 500–3000 ppm was 46.7, 69.9, 43.4 and 66.3%, respectively, as reflected in their TBA values. The mechanism by which this inhibition takes place is thought to be related to chelation of free iron which is released from hemoproteins of meat during heat processing. This would in turn inhibit the catalytic activity of iron ions. These results were further confirmed by Li *et al.* [100] who added 3000 ppm *N*-carboxymethylchitosan to cooked pork and found that this amount was sufficient to prevent the oxidative rancidity of the product. The feasibility of using chitosan powders in a fluorescence sensor for monitoring lipid oxidation in muscle foods was studied by Weist and Karel [101]. The primary amino groups of chitosan form a stable fluorosphere with volatile aldehydes such as malondialdehyde which is derived from the breakdown of fats.

Nutritional effect of chitin and chitosan in foods

Multiple action of chitin and chitosan in food systems relate to their effects as dietary fibre and as functional ingredients. The United States Food and Drug Administration (USFDA) approved chitosan as a feed additive in 1983 [69]. Chitosan is also used in the food industry as a food quality enhancer in certain countries. Japan produces dietary cookies, potato chips and noodles enriched with chitosan because of its hypocholesterolemic effect [102]. Furthermore, vinegar products containing chitosan are manufactured and sold in Japan, again because of their cholesterol lowering ability [102].

Recently, Hirano *et al.* [103] demonstrated the nutritional significance of chitinous polymers in animals and indicated the effectiveness of chitin and chitosan as feed additives. Normal growth patterns were observed with hens and broilers fed <1.4 g of chitosan/kg of body weight per day for up to 239 days and with rabbits fed <0.8 g of chitosan/kg of body weight per day for the same period. Furthermore, the serum cholesterol and triacylglycerol values of rabbits, hens and broilers were kept low by feeding 2% chitosan, but they were not kept low by feeding 1% chitosan or 2% chitin. In a similar study, Razdan and Pettersson [104] observed increased high density lipoprotein (HDL) concentrations after feeding chitosan containing diet to broiler chicken. This could be attributed to enhanced reverse cholesterol transport in response to intestinal losses of dietary fats. The effect of chitin, chitosan and cellulose as dietary supplements on the growth of cultured red sea bream, Japaneese eel, and yellow tail has been investigated by Kono *et al.* [105]. The growth rate of all fishes fed with a 10% chitin supplement was the highest, thus indicating its applicability in feed. Feed efficiency in the red sea bream and Japaneese eel fed a 10% chitin supplemented

diet was also the highest. Austin *et al.* [106] reported the effect of chitin as a feed additive on the growth of bifidobacteria in the guts of chickens. Addition of chitin increased the growth of bifidobacteria which are important as they inhibit the growth of other types of microorganisms. Bifidobacteria also generate the lactase required for digestion of milk lactose. This may be of significance for humans and animals with lactose intolerance [69, 106]. Therefore, one might formulate a digestible and highly nutritious animal feed in conjunction with high lactose cheese whey [106]. Use of chitin as a functional ingredient in dough fermentation for bread has been reported by Knorr and Betschart [107, 108]. They found that the loaf volume of wheat bread increased when up to 2% microcrystalline chitin was included in the formulation.

The nondigestibility in the upper gastrointestinal tract, high viscosity, polymeric nature and high water binding properties, together with low water binding in the lower gastrointestinal tract, are all responsible for the effective hypocholesterolemic potential of dietary fibres [109, 110]. Chitosan shows most of these criteria and has a highly characteristic property in relation to other dietary plant fibres. Due to the ability of forming ionic bonds at low pH it can bind *in vitro* to different types of anions such as bile acids or free fatty acids [109]. Large proportions of these bound lipids are thus excreted. Bound triacylglycerols would escape hydrolysis by lipase, promoting the excretion of fatty materials including cholesterol, sterols and triacylglycerols [110, 111]. Inside the digestive tract, chitosan forms micelles with cholesterol, both endogenous and from dietary sources, in the alkaline fluids in the upper part of the intestine, resulting in the depression of absorption of dietary cholesterol and circulation of cholic acid to the liver. Because of the formation of cholic acid from blood cholesterol in the liver it tends to decrease blood cholesterol concentration. Large intestinal microbials which secrete chitinases can digest these micelles and hence the formed bile acids and sterols are excreted as free forms into faeces without absorption [112]. On the other hand, Deuchi *et al.* [113] proposed that chitosan is solubilized in the stomach to form an emulsion with intragastric oil droplets and begins to precipitate in the small intestine at pH 6–6.5. With the aggregation of polysaccharide chains, the oil droplets are entrapped in their matrices thereby passing through the lumen and emptying into faeces.

Immobilization of enzymes by chitin and chitosan

Enzyme immobilization is a method to keep enzyme molecules confined in a distinct phase separated from the bulk phase while allowing exchange between these two phases [114]. Different methods such as covalent bonding, electrostatic bonding, copolymerization, polymer entrapment, hydrophobic interaction, liposomal entrapment and encapsulation are often used for immobilization of enzymes. The most common method is the covalent bonding onto an insoluble polymer such as cellulose and chitin. Immobilized enzymes are reusable, stable and suitable as specific industrial catalysts [114–116].

Immobilization of enzymes, namely α-amylase, β-amylase, glucose isomerase and amyloglucosidase on krill chitin activated by formaldehyde was studied by Synowiecki *et al.* [117] who documented possible mechanisms for immobilization of these enzymes. They proposed that the reaction was initiated by generation of the hydrated form of formaldehyde which condenses with free NH_2 groups of chitin, forming Schiff's bases and dihydroxymethyl derivatives of aldehyde. These Schiff's bases might be responsible for immobilization of enzymes by reacting with various functional groups of the enzymes, thus forming methylene bridges. A similar study by Han and Shahidi [118] reported 20–29% activity retention of crude seal gastric proteases after immobilization on glutaraldehyde-treated chitin. The characteristics of the immobilized crude native seal gastric proteases were similar to those of chymosin. The immobilization of penicillin G acylase on different physical forms of chitosan, namely beads, particles and powder was studied by Braun *et al.* [115] who observed activity retention of 40, 93 and 100%, respectively. Another study by Siso *et al.* [116] demonstrated that microencapsulation in chitosan beads was an effective enzyme immobilization method for invertase and α-amylase.

Other applications of chitinous materials in the food industry

Bioconversion of chitin to single cell protein

Bioconversion of shellfish wastes to single cell protein, which is a suitable feed supplement for animals and aquatic organisms, has been described [15, 16]. This process can play an economical role not only in shellfish processing, but also in integrated aquaculture systems [16]. Indirectly, it serves as a method of waste management which may reduce large quantities of shellfish waste from processing plants. There are four steps in production of single cell protein from shellfish waste [15]. These are; (I) drying, size reduction and chemical purification of shellfish processing waste; (II) extraction of chitinase enzyme from purified waste; (III) chitin hydrolysis; and (IV) fermentation in submerged culture (product generation stage).

These researchers further studied the appropriate microorganism for extracellular chitinase production and concluded that among *Serratia marcescens* W200, *Serratia marcescens* QMB 1466, *Serratia marcescens* 2875-ICPB, *Enterobacter liquefaciens* 3354-ICPB and *Aeromonas liquefaciens* 2327-ICPB, *Serratia marcescens* QMB 1466 was the most suitable candidate. Cosio *et al.* [119] found that a temperature of 30°C and an initial

pH of 7.5 in the medium were suitable for chitinase production of the above species.

According to the findings of Revah-Moiseev and Carroad [16], out of different yeast cultures, *Pichia kudriavzevii* was comparatively a better culture for production of single cell proteins under submerged fermentation condition than *Candida krusei* 57-19 and *Candida krusei* 61-287. They further concluded that *Pichia kudriavzevii* may be successfully grown in a chitin hydrolysis product at high temperatures and low pH values with an end product containing an acceptable amino acid distribution. According to the economic analysis of the shrimp shell bioconversion process by Cosio *et al*. [119], 6069 kg/day of shell waste can produce 68 kg/day of dry yeast (40% protein, 94%solids). In a similar study, Patil and Patil [120] also observed that a molasses fermentation medium supplemented with 0.25% chitin increased the yield of *Saccharomyces* yeast cell mass compared with yeast extract supplemented and the control without supplementation.

Production of ethanol from cane molasses

Patil and Patil [120] studied the possibility of accelerating the rate of ethanol production by supplementation with 0.2% carbohydrates such as acacia gum, chitin, xylan, pullulan, cellobiose, dextrin, inulin and agar. They used two yeast strains *Saccharomyces cerevisiae* NCIM 3526 and *Saccharomyces uvarum* NCIM 3509 for all fermentation reactions and found that, among carbohydrate supplements used, chitin was most effective in accelerating the rate of ethanol production from cane molasses.

Approximately 5.38–5.60% ethanol was formed after 36 h at 37°C from cane molasses containing 16% reducing sugar with chitin supplements (0.2%) in the fermentation medium. In the absence of any supplement, more than 72 h was needed to produce the same amount of ethanol. Chitin supplementation can reduce the fermentation time to one-third and hence the cost of ethanol production can be reduced. Patil and Patil [120] also demonstrated that the rate of ethanol formation was enhanced in the presence of chitin, acacia gum, xylan, dextrin or cellobiose in the broth culture media. In the same study, these researchers further observed that the rate of ethanol production was higher for all the culture strains used in the presence of chitin than simply with yeast extract supplements or controls with no supplements.

Preparation of chitin and chitosan oligomers and their applications in food for human health

Research on preparation and physiological activities of chitin and chitosan oligomers has continuously attracted much attention in the food and pharmaceutical fields due to their versatile antitumor activity [121, 122], immuno-enhancing effects [123, 124], protective effects against some infectious pathogens in mice [125, 126], antifungal activity [58, 127], and antimicrobial activity [58, 128].

There are two hydrolytic methods, to prepare chitin and chitosan oligomers: acid hydrolysis and enzymatic hydrolysis. Acid hydrolysis with inorganic acids leads to the formation of oligomers with a low degree of polymerization (DP), varying from monomer to trimer in quantitative yield. Then, the yield of oligomers with relatively higher DP, such tetramer to heptamer, which are desirable as biologically active oligomers, is low. In contrast to acid hydrolysis, enzymatic hydrolysis of chitin and chitosan by chitinase, chitosanase, lysozyme, and cellulase readily allows production of high DP oligomers for different applications. A detailed description of preparation methods for chitin chitosan oligomers is available in the existing literature [129–139].

Studies on functional properties of chitin and chitosan oligomers have clearly revealed their high dependency on the degree of polymerization [121, 58]. The oligomers of high DP from pentamer to heptamer had a better characteristic functionality in comparison with the relatively low DP oligomers [140].

Studies have shown the antitumorigenic properties of chitin and chitosan oligomers in the inhibition of the growth of tumor cells via an immuno-enhancing effect [121]. Suzuki *et al*. [141] also revealed that *N*-acetylchitooligosaccharides, from $(GlcNAc)_4$ to $(GlcNAc)_7$, displayed strong attracting responses to peritoneal exudate cells in BALB/c mice, whereas chitooligosaccharides, from $(GlcN)_2$ to $(GlcN)_6$, did not exhibit such an effect. Suzuki *et al*. [142] also found that chitin and chitosan oligomers, $(GlcNAc)_6$ and $(GlcN)_6$, had a tumor growth-inhibitory effect in allogenic and syngeneic mouse system, including sarcoma 180 solid tumor and MM46 solid tumor. Further, it was concluded that the effect was host-mediated and not by direct cytocidal action on the tumor cells. Tokoro *et al.* [124] showed that the two oligosaccharides, $(GlcNAc)_6$ and $(GlcN)_6$, exhibit growth-inhibitory effect against Meth-A solid tumor transplanted into BALB/c mice and the antitumor mechanism was assumed to involve increased production of lymphokines, including interleukins 1 and 2, sequentially, leading to the manifestation of antitumor effect through proliferation of cytolytic T-lymphocytes. Tsukada *et al*. [122] reported a significant antimetastatic effect for $(GlcNAc)_6$ in mice bearing Lewis lung carcinoma. Suzuki *et al*. [123] analyzed the change of the spleen cells from tumor-bearing mice administered with chitooligosacchairde such as $(GlcNAc)_6$ to unravel the tumor inhibitory mechanism and cell growth by immuno-enhancing effects of the oligomers. It was demonstrated that increase of cytotoxic T lymphocytes activity by accelerating the differentiation of helper T cells was remarkable and paralleled a decrease of suppressor T cells activity.

On the other hand, chitin and chitosan oligomers

were responsible for enhancing protective effects against infection with some pathogens in mice. Tokoro *et al.* [126] demonstrated the protective effect of chitin oligomer in mice infected with *Listeria monocytogenes*, based on the fact that interferon-λ and interleukin 2 were able to enhance the growth-inhibitory effect on *L. monocytogenes* by $(GlcNAc)_6$-treated macrophages. Yamada *et al.* [125] showed that $(GlcNAc)_6$ induced phytoalexin formation in suspension-cultured rice cells, and GlcNAc oligomers smaller than trimers and a series of deacetylated oligomers had almost no activity.

Chitosan oligomers as well as chitosan have been shown to inhibit growth of several fungi and bacteria, especially pathogens [128, 139]. Hirano and Nagao [58] have studied the relationship between the degree of polymerization of chitosan and the inhibition effect. They showed that chitosan oligomers (DP 2-8) as well as low-molecular-weight chitosan possessed stronger growth inhibitory effect than high-molecular-weight chitosan against several phytopathogens including *Fusarium oxyporum*, *Phomopsis fukushi*, *Alternaria alternata*, among others. Kendra *et al.* [127] explained that some of chitosan oligomers with biological activity, present in the interface of pea/*Fusarium* appear to inhibit the fungal growth. Uchida *et al.* [128] found that the oligomers with higher molecular weight, which were slightly hydrolyzed with chitosanase, were more active in both antifungal and antibacterial activities than native chitosan and lower molecular weight oligomers. Jeon and Kim [139] reported that, among the three fraction of oligomers produced and separated using ultrafiltration membrane enzymatic reactor system, the highest molecular weight oligomers (MW 5000–10,000 Da) had the strongest bactericidal and fungicidal activities against most pathogens tested. Muraki and Aiba [143] has shown that partially derivatized *N*-lauroyl (PNL) chitooligosaccharides (DP 7-8), PNL-$(GlcN)_7$ and PNL-$(GlcN)_8$, with the degree of *N*-lauroylation of about 50%, had fairly strong antibacterial activity against the growth of *E. coli*, compared to all $(GlcN)_n$ and PNL-$(GlcN)_n$ with a chain length smaller than seven residues.

Conclusion

Even though chitin, chitosan and their derivatives have been considered as versatile biopolymers in food applications their potential uses as functional food ingredients have to be studied with broader emphasis. In that sense, research and development should have great potential in finding novel uses in product development, microbiology, edible film industry, water purification, purification of waste discharge from food processing waste and nutritional aspects related to chitin, chitosan and their derivatives.

Most physiological activities and functional properties of chitin and chitosan oligomers clearly depend upon their molecular weights and that a chain length of at least five residues is required. These oligomers may be more advantageous than chitin and chitosan as polymers in the field of food additives and nutraceuticals in human health, because chitin and chitosan could not be degraded in the human intestine due to the absence of enzymes such as chitinase and chitosanase. In this context, chitin and chitosan may behave as dietary fibres which are excreted without any degradation in the intestine.

Furthermore, it is a current matter of discussion as to whether these biopolymers may have the potential to influence physiological functions or metabolism in the human body. Therefore, a significant increase in the number of scientific studies to obtain evidence to support any health or performance claim can be expected. In that sense, further detailed physiological and sensory studies are required to determine the mechanisms of these effects and, ultimately, to come to a better understanding of how they may be manipulated in the creation of better quality foods.

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