Chitosan as an antimicrobial agent

Despite certain hurdles, such as limited efficacy and legislative problems, chitosan has enormous potential to improve the quality and safety of our food.

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Throughout history, mankind has been striving to improve the safety and stability of food. What began in an empirical way with a few simple processes such as smoking and salting is now a precise science, and modern food technologists have a wide range of preservative compounds at their disposal. Despite the undoubted benefits, however, there are concerns over the long-term health effects of many food preservatives in use today. Modern consumers are increasingly concerned about the health implications (be they real or imagined) of the foods they eat, as evidenced by the growing trend for additive-free and organic produce.

There is, thus, both a commercial and a public health incentive to develop novel natural antimicrobial systems for use in foods and food-related applications, and to reduce dependence on traditional preservatives. Worldwide, considerable research effort is dedicated to achieving this objective.

Wide range of applications

Chitosan is a ß-1,4-linked polymer of glucosamine (2-amino-2-deoxy-ß-D-glucose) and lesser amounts of *N*-acetylglucosamine. It is formed by the deacetylation of chitin (poly-*N*-acetylglucosamine), an abundant byproduct of the crab and shrimp processing industries. The distinction between chitin and chitosan is somewhat blurred; some maintain that chitin that is more than 50 per cent deacetylated is chitosan, whereas others define chitosan as soluble in 1 per cent acetic acid, chitin being insoluble[1]. Most commercially available chitosan preparations are more than 85 per cent deacetylated, and have molecular weights between 100kDa and 1000kDa. They are usually complexed with acids, such as acetic or lactic acids.

An important property of chitosan is its positive charge in acidic solution. This is due to the presence of primary amines on the molecule that bind protons according to the equation:

$$\text{Chit-NH}_2 + \text{H}_3\text{O+} \Leftrightarrow \text{Chit-NH}_3\text{+} + \text{H}_2\text{O}$$

The pKa value for the equation is approximately 6.3. Chitosan solubilises when more than 50 per cent of the amino groups are protonated[2], so the solubility of most chitosan preparations decreases sharply as the solution pH rises above 6.0-6.5[3]. The maximum soluble concentration varies with different chitosans, but is usually around 10-20g L^{-1}. Chitosan solutions have good film-forming properties and are therefore potentially useful in gels and coatings. Because of its abundance, low cost and relatively unusual properties, chitosan and its derivitives have been used, at least at the experimental level, in a diverse range of applications.

Antimicrobial activity

Chitosan is antimicrobial against a wide range of target organisms. Activity varies considerable with the type of chitosan, the target organism and the environment in which it is applied. Consequently, literature reports vary somewhat and are, occasionally, contradictory. But generally speaking, yeasts and moulds are the most sensitive group, followed by Gram-positive bacteria and finally Gram-negative bacteria.

Experimental work with the baker's yeast *Saccharomyces cerevisiae* showed that fermentation was halted by as little as 3.6mg L^{-1} chitosan in a buffer system[4]. Similar powerful activity has been demonstrated against the mould *Fusarium solani,* the growth of which was prevented by 4mg L^{-1} chitosan in a liquid nutrient medium[5]. Variation in sensitivity between closely related micoorganisms was illustrated in an experiment in which phytopathogenic fungi were screened for sensitivity to chitosan in liquid media[6]. One *Cytosporina* sp. isolate was completely inhibited by 75mg L^{-1} chitosan, while a second isolate of the same genus was unaffected by 1000mg L^{-1}.

There are several factors, both intrinsic and extrinsic, that affect the antimicrobial activity of chitosan. It has been demonstrated that lower molecular weight chitosans (of less than 10kDa) have greater antimicrobial activity than native chitosans[7]. However, a degree of polymerisation of at least seven is required; lower molecular weight fractions have little or no activity[4,7]. Highly deacetylated chitosans are more antimicrobial than those with a higher proportion of acetylated amino groups, due to increased solubility and higher charge density[8].

Lower pH increases the antimicrobial activity of chitosan for much the same reasons, in addition to the 'hurdle effect' of inflicting acid stress on the target organisms. Temperature also has an effect, as — not ideally for many food applications — higher temperature (37°C) has been shown to enhance antimicrobial activity compared to refrigeration temperatures[9]. However, the greatest single influence on antimicrobial activity is the surrounding matrix. Being cationic, chitosan has the potential to bind to many different food components such as alginates, pectins, proteins and inorganic polyelectrolytes such as polyphosphate[10]. Solubility can be decreased by using high concentrations of low molecular weight electrolytes such as sodium halides, sodium phosphate and organic anions[11]. These factors mean that, as with most preservative systems, promising results obtained *in vitro* in buffer or microbiological media do not necessarily translate well into real food systems.

Food applications

The physico-chemical properties of chitosan described above obviously dictate, to a large extent, the food applications for which it would be suitable. Many applications are as a surface coating on meat products and fruits, or as an additive to acidic foods.

A perusal of the European Patent Office database reveals over 2000 patents involving chitosan, but only a few of these concern food applications. A Russian patent (RU2170022; 2001) describes the use of up to 0.1 per cent w/w chitosan in combination with 0.1 per cent sorbic acid and a third antibacterial agent as a preservative and antioxidant in caviar. The applicants claim that the product shelf life is extended by the use of the specified preservative agents.

An earlier patent by a Japanese company (US5549919; 1996) describes the addition of one, or more, of chitosan, protamine and lysozyme to foods in a sealed package containing a deoxidising agent. It is claimed that microbial degradation of the food is thus prevented to a greater extent than can be achieved by conventional preservation systems.

The incorporation of chitosan in an antimicrobial film at up to 20 per cent w/w together with an anionic or non-ionic surfactant is described by patent JP11032742 (1999). The film is intended for use in packaging materials for foods, medical supplies and so on, or as a laminated coating on items for which surface colonisation is undesirable.

At the experimental level, there are numerous papers describing the application of chitosan as a preservative in foods. For example, dipping fresh UK pork sausages in 1 per cent chitosan glutamate solution prior to storage at 7°C inhibited microbial growth and was shown to increase the shelf life by eight days[12]. The coating technique has also been applied to fruits. In one trial, coating strawberries and raspberries with 2.0 per cent w/v chitosan was almost as effective as the fungicide TBZ at preventing spoilage during storage at 13°C[13]. Loss of colour, wilting and fungal infection in cucumber and bell pepper fruit were all improved by coating with a 1.5 per cent chitosan solution[14].

To improve the antimicrobial action of chitosan films, other preservatives can be incorporated, such as organic acids. However, chitosan addition does not always result in a beneficial extension of shelf life. In many experimental reports, an initial decrease in microbial counts is seen, followed by a rapid outgrowth of resistant strains such that by the time the normal shelf life is reached, the total counts in the control and chitosan-treated samples are similar.

Possible problems

Legislative issues present some difficulty to the food technologist wishing to exploit chitosan as a food preservative. Although it is permitted as an additive in Japan, for example, it is only permitted as a processing aid in most Western countries.

Another issue that has been poorly researched is the impact of dietary chitosan on human health. It is already marketed in capsule form as a slimming aid on the basis that it binds to fats in the digestive tract and prevents their adsorption. This raises the question of whether it binds to anything else, such as micronutrients and trace elements. Chitosan reaching the colon could also have an adverse affect on the gut flora composition.

Great potential

Despite certain difficulties and the limited efficacy of chitosan as a preservative, it is still an interesting compound with considerable potential for improving the quality and safety of our food. The most successful applications are likely to be those in which specific problems are targeted, such as *Botrytis cinerea* infestation of strawberries, or those in which chitosan forms part of a multicomponent 'hurdle' system of preservation. ❖

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