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Chitosan-based dietary foods

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Chitosan exhibits anticholesterolemic, antiulcer and antiuricemic properties when orally administered, and is potentially suitable for the prevention of the coeliac disease. This set of properties stems from the capacity to bind specifically fatty acids, bile acids, phospholipids, uric acid and the toxic gliadin fraction. Chitosan does not depress the serum iron and hemoglobin, does not overstimulate the 3-hydroxy-3-methylglutaryl CoA reductase; it does not alter the human intestinal microbiota, but lowers the putrefaction metabolites. Its susceptibility to the hydrolytic action of lipases is discussed. For oral administration, chitosan should be supplied with a proper degree of polymerization and chemical form. Copyright © 1996 Published by Elsevier Science Ltd

INTRODUCTION

Chitosans, a family of polysaccharides derived from crustacean or fungal chitins (Fig. 1), are of particular interest in the food area. They are the only abundant aminated polysaccharides, and their cationicity has attracted interest for a number of purposes (Muzzarelli, 1993, 1996; Muzzarelli *et al.*, 1986). Their beneficial actions on the human body are well documented, and, as far as the title subject is concerned, the basicity of the amino group, which in early times directed attention to the antiulcer activity of chitosan (Doczi *et al.*, 1964; Hillyard *et al.*, 1964; Weisber & Gubner, 1966), is the key factor for the biological significance of this dietary fiber, which is expected to be useful in the *per os* treatment of arthrosis and osteoporosis as well (Ito, 1995). The chitosan scavenging action for chloride ions also prevents blood pressure elevation via control of the angiotensin converting enzymes (Okuda, 1995).

The inclusion of polyaminosaccharides in the list of dietary fibers was delayed by the fact that they are not of plant origin. Edible fibers, however are polysaccharides, related polymers and lignin, which are resistant to hydrolysis by the digestive enzymes of man. The potential of chitosan as dietary fiber was discussed by Furda (1983), Ishikura (1993) and by Furda and Brine (1990). Hypocholesterolemic quaternary ammonium salts of chitosan were also described by Chandler and Curatolo (1992).

The scope of the present work is to highlight the most recent advances, especially in consideration of the granted approval of chitin and chitosan as food additives in Italy, Norway and Japan (Tsugita & Sakamoto, 1995; Wakayama *et al.*, 1996).

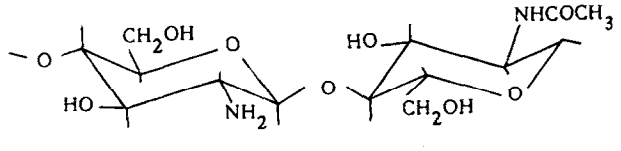

Fig. 1. Segment of the polysaccharide chain showing β(1-4) linked *N*-acetylglucosamine and glucosamine units. Chitosans are copolymers of these two compounds in various proportions and in random sequences: in most industrial chitosans the degree of acetylation (molar ratio *N*-acetylglucosamine/all units) is close to 0.2.

HYPOCHOLESTEROLEMIC ACTION ON ANIMALS

The hypocholesterolemic potential of a dietary fiber resides in high viscosity, polymeric nature and high water-binding properties and nondigestibility in the upper gastrointestinal tract, together with low water binding in the lower gastrointestinal tract. Chitosan meets most of these criteria, and has a highly characteristic property in relation to other dietary plant fibers: it can bind anions such as bile acids or free fatty acids at low pH by ionic bonds resulting from its amino group.

Chitosan binds fatty acids to form the corresponding salts (Ahmad *et al.*, 1995). The binding is mainly of ionic nature: in fact the salt is prepared by neutralization of chitosan with edible fatty acids such as oleic, linoleic, palmitic, stearic and linolenic acids. The resulting salts,

after ingestion bind additional lipids, probably due to hydrophobic interactions (triglicerides, fatty and bile acids, cholesterol and other sterols) and a great portion of these bound lipids are excreted rather than absorbed. Hydrochloric acid in the stomach would not hydrolyse chitosan fatty acid salts because this material would not wet. This chitinous material would grow in size as it travels through the gastrointestinal tract, and would bind additional amounts of lipids. Bound triglycerides would escape hydrolysis by lipase, promoting the excretion of fatty materials including cholesterol, sterols and triglicerides. (Furda, 1980; Zacour *et al.*, 1992).

Chitosan was compared to cholestyramine, an anionic synthetic resin (Dowex A-1) which is being used to sequester phospholipids, monoglycerides, fatty acids and cholesterol. This resin might interfere with lipid absorption not only by removal of amphipathic micellar components such as bile acids and phospholipids, but also by sequestration of cholesterol and hydrolysis products of triglicerides. Cholestyramine is currently used to treat hypercholesterolemia in humans. Although very effective, its use has been questioned because of reports of a possible link to colon cancer in humans and rat experimental models. Cholestyramine sequesters bile salts in rats and alters intestinal morphology in humans. Furthermore there is a significant increase in rat intestinal tumor induction by various agents when cholestyramine is added to the diet. DEAE-Dextran was also found effective in reducing intestinal fat absorption and speeding up bile turnover (Pupita & Barone, 1987).

Lipid absorption depression

Chitosan has viscous properties more like those of viscous dietary fibers such as pectin and guar gum. The viscous fibers also sequester micellar components *in vitro*, albeit with considerably less avidity than does cholestyramine and other commercial anion-exchangers. This is likely due to a trapping in the gel matrix, and may readily dissociate under *in vivo* conditions (Vahouni *et al.*, 1983).

The mechanisms by which cholestyramine and chitosan affect lipids absorption may by different. Sugano *et al.* (1988) have reported that after a 20-day feeding of 5% cholestyramine or chitosan to rats on a cholesterol-containing diet, plasma and liver cholesterol levels in both groups were significantly lower than in controls. However, fecal neutral sterols were elevated only in the chitosan-fed rats, suggesting a difference in the action of chitosan and cholestyramine. In a subsequent study, the feeding of chitosan caused increased fecal output of neutral sterols but not acidic steroids, while cholestyramine feeding increased mainly fecal acidic steroids. The data of Sugano *et al.* (1988) also suggest that the mechanism of chitosan action may not completely parallel those of the viscous dietary fibers. Pectins, when fed at sufficient levels, cause some increase in the output of fecal neutral and acidic steroids, but this appears insufficient to account for their hypocholesterolemic effects. However, chitosan, which has greater hypocholesterolemic potency than pectins in cholesterol-fed rats does not effectively increase bile acid excretion.

The data obtained by Fukada *et al.* (1991) indicate that chitosans affect the metabolism of intestinal bile acids in rats. Thus, based on evidence derived from analyses of fecal acidic and neutral steroid output, one cannot easily assign a common mechanism of action for cholestyramine, chitosan, and viscous dietary fibers.

Characteristics of chitosans and other polymers

The degree of acetylation and the degree of polymerization are two important parameters used to characterize chitosans. They influence the viscosity of the solutions of chitosan salts and have been shown to have prominent roles in the biochemical significance of chitosan. Therefore, the relationship between hypocholesterolemic efficacy and average molecular weight of chitosan was studied in rats fed a cholesterol-enriched (0.5%) diet by Sugano *et al.* (1988). Several chitosan preparations with a comparable degree of deacetylation but differing widely in average molecular weight and viscosity, almost completely prevented the rise of serum cholesterol at the 5% dietary level. At the 2% level, chitosans with viscosities at both extremes exerted a comparable cholesterol-lowering action. The hypocholesterolemic action of chitosans is independent of their molecular weight within the tested range. Judging from the ineffectiveness of the glucosamine, it was suggested that some degree of polymerization is required to provoke a cholesterol-lowering activity (Sugano *et al.*, 1992).

The concentration of serum cholesterol at day 7 was significantly lower in rats fed chitosan preparations with MW 5000–50,000 Da than in those fed cellulose. The 2000 Da chitosan preparation did not show a hypocholesterolemic effect at day 7. All chitosan hydrolysates except for 2000 Da significantly increased fecal excretion of neutral steroids as cholesterol and coprostanol. The hydrolysates with average molecular weights above 10,000 Da were more effective in enhancing fecal excretion of neutral steroids (Ikeda *et al.*, 1993). Enomoto *et al.* (1992) noted that chitosan having ca. 8000 was more effective in lowering cholesterol than chitosan having 220,000. The effect on the composition of neutral steroids was diverse depending on the preparations. Excretion of total acidic steroids was slightly increased in rats fed chitosan hydrolysates except for the 2000 Da group.

Deuchi *et al.* (1994) investigated the effects of various dietary fibers on the fat digestibility by rats fed on a high-fat diet. Each of 23 different fibers was added (5% w/w) to a purified diet containing corn oil (20% w/w). The rats were fed these diets for 2 weeks, and the feces were collected from each animal during the last 3 days. When compared with cellulose (control), 10 of the

tested fibers significantly increased the fecal lipid excretion. Chitosan markedly increased the fecal lipid excretion and reduced the apparent fat digestibility to about a half relative to the control. The fatty acid composition of the fecal lipids closely reflected that of the dietary fat. These results suggest that chitosan has a potential for interfering with fat digestion and absorption in the intestinal tract, and for facilitating the excretion of dietary fat into the feces.

A soluble form of chitosan would be able to interfere with intraluminal lipid absorption through the interaction with micelle formation or emulsification of lipids in the enteric phase. Nauss *et al.* (1983) and Ikeda *et al.* (1993) have offered similar suggestions on the basis of their studies on fully emulsified oil with chitosan and sodium taurocholate. It has also been suggested that dietary fiber would not necessarily limit the *in vivo* absorption. It is therefore noteworthy that chitosan had a strong ability to increase the fecal lipid excretion *in vivo*.

Deuchi *et al.* (1994) consider that the chitosan dissolves in the stomach to form an emulsion with intragastric oil droplets and begins to precipitate in the small intestine at pH 6.0–6.5. As the polysaccharides chains start to aggregate, they would entrap fine oil droplets in their matrices, pass through the lumen and empty into the feces.

Synergistic action of ascorbate

Rats were fed on high-fat diets containing cellulose (control), chitosan, chitosan ascorbate, chitosan lactate and chitosan citrate salts. The presence of ascorbate caused a larger increase in the fecal fat excretion than otherwise, without considerably affecting the apparent protein digestibility (Fig. 2). The mechanism for depressing fat digestibility by chitosan ascorbate was explained in the following terms: gastric acid-soluble chitosan is mixed with dietary fat in the stomach, the emulsifying process being effectively mediated by ascorbic acid. When the digest emptied from the stomach comes into contact with pancreatic juice (in an alkaline pH range), oil droplets become embedded in the gelled chitosan matrices and are excreted into the feces without fully undergoing absorption. Kanauchi *et al.* (1994) carried out similar studies with 7% chitosan diet and reduced fat absorption to one third of the controls within one month.

The mechanism for the synergistic effect is considered by Kanauchi *et al.* (1995) to be (a) viscosity reduction in the stomach, (b) increase of the oil-holding capacity of the chitosan gel, and (c) reduced leaking of entrapped fat in the intestinal tract, the resulting is chitosan-fat gel more flexible. A scheme is provided in Fig. 3.

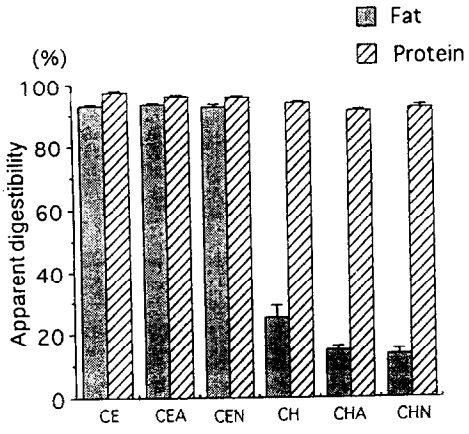

Fig. 2. Apparent digestibility of fats and proteins by rats fed with cellulose or chitosan. The apparent fat digestibility was calculated as 100 [(ingested lipids − fecal lipids) / ingested lipids]. Abbreviations: CE, cellulose; CEA, cellulose with ascorbic acid; CEN, cellulose with sodium ascorbate; CH, chitosan; CHA, chitosan with ascorbic acid; CHN, chitosan with sodium ascorbate.

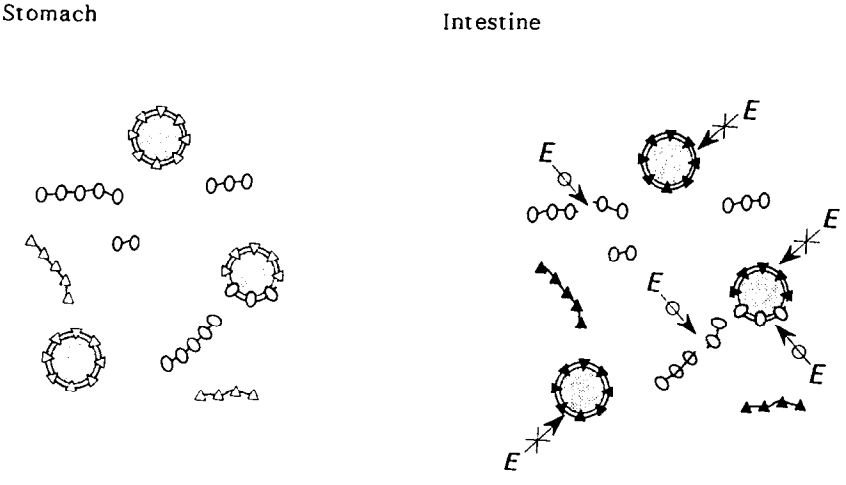

Fig. 3. Schematic illustration of the chitosan-promoted inhibition of fat digestion. In the stomach (left), chitosan dissolves in an acidic medium and emulsifies fats; in the intestine (right), a change in the chitosan gel form occurs and the entrapped oil droplets are not attacked by pancreatic or intestinal enzymes. Oil droplets emulsified with casein are digested. Triangles = chitosan; Circles = casein; *E* = digestive enzymes.

Chitosan also affected the growth rate by reducing the energy intake by the inhibition of lipid digestibility. About 80% of dietary fat could not be digested by supplementing chitosan with ascorbic acid, so the rats could not intake sufficient energy when compared to the control animals. Despite feeding with a high-fat diet, the plasma triglycerides concentration and epididymal fat pad weight in the chitosan-receiving groups remained at low levels, probably because of a depressed fat intake (Kanauchi *et al.*, 1994).

The protein digestibility in rats fed with the chitosan diets was not markedly changed. This recent finding (Deuchi *et al.*, 1994; Kanauchi *et al.*, 1994) could possibly be explained in the light of the effect of chitosan on the permeability of monolayers of intestinal epithelial cells (Artursson *et al.*, 1994). Because of their positive charge, cationic macromolecules such as protamine, polylysine and chitosan can interact with the anionic components (sialic acid) of the glycoproteins on the surface of the epithelial cells. In a similar study, an intravenous infusion of protamine produced large increases in lymph flow and in lymph protein clearance. This enhanced transcapillary protein flux was explained to be primarily due to the neutralisation of fixed anionic sites on the capillary wall. Further, it has been suggested that cationic macromolecules are able to displace cations from electronegative sites (such as tight junctions) on a membrane which require coordination with cations for dimensional stability.

The liposoluble vitamin E status in rats fed on a high fat diet was examined by Deuchi *et al.* (1995) who found a decrease of serum vitamin E level accompanied by a decline of calcium, over a 2-week period of chitosan ascorbate administration.

HYPOCHOLESTEROLEMIC EFFECT OF CHITOSAN IN HUMANS

During the last few years, pre-clinical and clinical studies have been published. Maezaki *et al.* (1993) studied the dietary effects of chitosan in adult males. The chitosan was administered in the form of biscuits over a period of 4 weeks. When chitosan was given in the diet (3–6 g/day), the serum total cholesterol level significantly decreased, while the serum HDL-cholesterol level significantly increased when compared with the level for each of them before ingestion. Similar results have been obtained by Sugano *et al.* (1988) with rats raised for 20 days after dietary administration of chitosan. The results of Maezaki *et al.* (1993) on humans show a favorable effect with a very low dose within a short period.

The chitosan intake (biscuits) by healthy volunteers was found by Terada *et al.* (1995) to produce a significant decrease of the fecal phenols, *p*-cresol and indole, in analogy with other polysaccharides. Chitosan inhibited the putrefactive activity of the intestinal microbiota thus reducing the risks of disease states. The lecithinase negative clostridia were the only microbiota significantly depressed by chitosan in humans, after 2-week chitosan intake period.

Whilst biscuit cooking imparts appealing organoleptic properties, the Maillard reaction would partly destroy the primary amino groups of chitosan and reduce its efficacy (Tanaka *et al.*, 1993). Biscuits were also manufactured by Sakamoto et al. (1991). This fact should be taken into account when baked products are proposed. The chitosan intended for oral administration should preferably not be submitted to thermal treatment such as cooking and baking. The determination of chitosan in foods is feasible according to Maesaki and Yamazaki (1993).

Clinical studies were in fact based on the administration of chitosan in the form of tablets, i.e. prepared with no thermal treatment.

In a randomized, double-blind, placebo-controlled trial, the body weight loss and the lipid-lowering effects obtained with a caloric restriction and a new dietary fiber (a mixture of chitosan, guar's meal, ascorbic acid and others micronutrients) were investigated by Veneroni *et al.* (1996) in a weight reducing program of 80 obese adult subjects with hyperlipidemia. Subjects were treated with hypocaloric diet plus 4 tablets/day of chitosan dietary fiber or with hypocaloric diet plus 4 tablets/day of placebo for 4 weeks. At the end of the study period a statistically significant reduction in the body weight and overweight, triglycerides and total and LDL cholesterol, and an augmentation of HDL cholesterol were observed in both groups but in the chitosan treated group the differences were statistically greater than in the placebo group (Table 1).

Body weight reduction was 7.9 kg in the chitosan group and 3.4 kg in the placebo group; overweight reduction was 11.2% in the chitosan group and 4.2% in the placebo group; total cholesterol reduction was 23.9% in the chitosan group and 10.4% in the placebo group; LDL cholesterol reduction was 33.4% in the chitosan group and 12.1% in the placebo group; triglycerides reduction was 23.5% in the chitosan group and 9.3% in the placebo group; HDL cholesterol augmentation was 10.2% in the chitosan group and 3.5% in the placebo group. No pathologic or clinically significant change in blood chemistry or hematological data were observed.

Ventura (1996) conducted a research programme to determine the dose effect in lipid lowering activity of a new dietary integrator (tablets containing chitosan, 240 mg, *Garcinia cambogia* extract, 55 mg, and chromium, 19 mg) in a weight-reducing programme of 150 obese subjects. The study was a randomized, double blind, placebo controlled trial, in which the subjects were treated with hypocalorid diet plus two tablets/day of placebo (50 subjects) or with hypocalorid diet plus

Table 1. Hematic parameters during the chitosan treatment

Parameter	plasmatic conc. after 4 weeks diet + chitosan	plasmatic conc. after 4 weeks diet + placebo	% difference after 4 weeks diet + chitosan	% difference after 4 weeks diet + placebo	treatment statistical comparison°
Total cholesterol	241.5±30.7*	272.8±29.8*	−23.9±3.8*	−10.4±6.2*	$p < 0.01$
HDL cholesterol	27.4±9.9*	26.6±9.1*	+10.2±7.1*	+3.5±2.6*	$p < 0.01$
LDL cholesterol	134.7±38.5*	173.4±39.2*	−33.4±9.2*	−12.1±6.5*	$p < 0.01$
Triglycerides	192.6±35.6*	216.4±37.4*	−23.5±5.3*	−9.3±3.6*	$p < 0.01$

* difference statistically significant in comparison with the baseline (Student's *t* test for paired data).
° Student's *t* test for independent data.
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two tablets/day of dietary integrator (50 subjects) for four weeks.

Overweight reduction was 12.5% (control 4.3%), total cholesterol reduction was 28.7% (control 10.7%), LDL cholesterol reduction was 35.1% (control 15.2%), triglycerides reduction was 26.6% (control 13.2%), HDL cholesterol augmentation was 11.1% (control 6.3%).

From the results obtained in these studies as well as those by Giustina and Ventura (1995) and Sciutto and Colombo (1995), the diet plus chitosan and guar fiber, or plus chitosan and *Garcinia* extract, appear to be useful treatments of overweight and hyperlipidemia in obese subjects.

POTENTIAL TREATMENT OF THE COELIAC DISEASE

The coeliac disease is an enteropathy occurring in genetically predisposed individuals; epidemiological investigations have been made by Catassi *et al.* (1994). Clinical studies have shown that rye, barley and probably oat, in addition to wheat, are toxic (Baker & Read, 1979; Weijers & Vande Kamer, 1955) whilst rice and maize are non toxic and are used safely in the coeliac patients diet. Toxicity of cereals other than wheat in coeliac disease is most likely due to prolamine fractions equivalent to gliadins (Auricchio *et al.*, 1984; Pittschieler *et al.*, 1994). The total peptic–triptic digest (PT-digest) of prolamine peptides from cereals, toxic in coeliac disease, agglutinates K562(S) cells and inhibits the development of the small intestine of 17-day old rat foetus and the improvement of cultured jejunal flat mucosa of coeliacs (Auricchio *et al.*, 1986). There is a strong correlation between the toxicity of cereals and the culture of the rat fetal intestine as well as the agglutinating activity on K562(S) cells. These biological activities probably related to the noxious effects of these peptides for the coeliac intestine, are all prevented by mannan (Auricchio *et al.*, 1990) and oligomers of *N*-acetylglucosamine (Auricchio *et al.*, 1987), suggesting that the agglutinating and toxic peptides are bound by these carbohydrates. Mannan or *N*-acetylglucosamine oligomers are able to bind all the peptides of a gliadin digest that agglutinate K562(S) cells and damage the fetal rat intestine *in vitro* (Auricchio *et al.*, 1993). Methylpyrrolidinone chitosan (MP-chitosan) coupled to Sepharose-6B was used to isolate hexaploid wheat peptides endowed of the agglutinating activity by De Vincenzi *et al.* (1993).

5-Methylpyrrolidinone chitosan was coupled to Sepharose-6B (Muzzarelli, 1992; Muzzarelli *et al.*, 1993). Enzymatic digests of the prolamine fractions from wheat (*Triticum aestivum*, var. S. Pastore), rye (*Secale cereale*, var 500-2G), barley (*Hordeum vulgare*, var. Arma) and oat (*Avena sativa*, var. Astra), were prepared according to De Ritis *et al.* (1979). Each digest (ca. 50 mg) was percolated at the flow-rate of 24 ml/hr through a methylpyrrolidinone chitosan – Sepharose-6B column (5×35 cm) equilibrated with 0.02 M ammonium acetate buffer, pH 7.2. Fractions A and B were collected and the column was washed with the above buffer until no absorbance at 278 nm was detected in the effluent. Fraction C was eluted from the column with 0.1 N acetic acid (introduced at eluant/bed volume ratio 1.6), after the pH value dropped to 2.87. Fraction C was immediately neutralized by addition of 0.5 M ammonium hydroxide (ca. 450 ml/tube).The agglutination test was based on the use of K562(S) cells resuspended with Dulbecco phosphate buffer saline at the concentration of 10^8 cells per ml. The fractions A and B tested at the concentration of 14 g/l of culture medium were not active in agglutinating K562(S) cells; on the contrary, fraction C (ca. 1.0–1.3% of the total prolamine loaded), showed higher agglutinating activity than the PT-digest itself. The minimum concentration required to agglutinate the totality of the cells in suspension was 1.6–25.0 mg per liter of culture medium.

The MP-chitosan was an effective inhibitor of the agglutination induced by the digest from bread wheat gliadin: in the presence of 2.333 g of bread wheat gliadin peptides per liter of cell suspension, MP-chitosan exerted 100% inhibition at the concentration of 2.3 g/l. When added to agglutinated cells, MP-chitosan was able to dissociate them: in practice, the cell layer formed after 20 min in the presence of wheat gliadin peptides, dissociated under the effect of added MP-chitosan at the

said concentration, and the cells regained their normal appearance.

It is known that plants synthesize a wide array of proteins capable of binding to affinity matrices based on chitin. Those proteins contain one or more chitin-binding domains whose affinity is not restricted to chitin but may extend to complex glycoconjugates. Therefore, certain modified chitosans may prove even more effective than chitin in binding to lectins from *Triticum aestivum*, *Hordeum vulgare*, *Oryza sativa* and *Secale cereale*, which share similarities according to immunological, biochemical and carbohydrate binding criteria (Raikhel *et al.*, 1993).

Further studies might lead to a better understanding of the selective interaction of gliadin and MP-chitosan, in view of the prevention of the coeliac disease.

HYPOURICEMIC EFFECT OF CHITIN AND CHITOSAN

Some evidence indicates that chitin and chitosan may be used in health food for the prevention and treatment of hyperuricemia. Male rats were fed with a feed supplemented with cellulose (control), chitin or chitosan, and the uric acid in blood and urine was measured. The animals fed with the addition of chitosan had much lower uric acid in blood and urine than those of control (Maekawa & Wada, 1990). Rats were administered chitosan together with adenine. Whilst the uric acid concentration increased four folds within 24 days in controls (adenine-treated rats), no increase was reported for the chitosan treated animals (Mita *et al.*, 1989).

Wada (1995) has reviewed the effect of dietary fiber intake on the digestion of nucleic acids, the inhibition of hyperuricemia by chitosan and the effect of chitosan intake on the metabolism of uric acid. The capability of chitosan to absorb urea from artificial gastric and intestinal juices was demonstrated by Jing *et al.* (1991) and Yamaguchi (1989).

CONCLUSIVE REMARKS

Upon ingestion, chitosan forms micelles with cholesterol and dietary cholesterol in the alkaline fluids in the upper part of the intestine, resulting in the depression of the absorption of dietary cholesterol and the circulation of cholic acid to the liver. Cholic acid is synthesized from blood cholesterol in the liver resulting in a decrease of blood cholesterol concentration. The micelles are digested by chitinases secreted by intestinal microorganisms in the large intestine, and bile acids and sterols are excreted as free forms into feces without absorption (Hirano & Akiyama, 1995).

The oral administration of chitosan has posed the following questions: (a) consequences on the amount of iron in the body; (b) influence on the intestinal flora; (c) effects on lipases and other enzymes; (d) most suitable forms for administration. All of them have been addressed by various authors.

The chelating ability of chitosan could represent a worry in terms of depletion of iron. Jennings *et al.* (1988) showed that chitosan in rats does not reduce serum iron or hemoglobin, and has lipid lowering effects equivalent to those of cholestyramine without producing similar deleterious effects upon the intestinal mucosa. This subject was also addressed by Gordon and Williford (1983) with similar conclusions. Breads containing chitosan-treated heme iron were also proposed by Chiba (1988).

An animal model was set up by Nelson *et al.* (1994) to study the influence of dietary fiber on bacterial translocation after burn injury: the animals which received chitosan had lower levels of bacteria in the cecum, mesenteric lymph nodes and liver, thus it was concluded that chitosan helped in reducing the bacterial translocation.

If chitosan is a substrate for human lipase, it might be possible that the relatively large amount of chitosan introduced with the diet would in part prevent lipases from hydrolysing the lipids. It is well known that 2-monoglycerides and fatty acids are the partial hydrolysis products, yielded by pancreatic lipase, suitable for absorption. Studies on the lipase–chitosan system are in progress, and include wheat germ lipase (Muzzarelli *et al.*, 1995), porcine pancreatic lipase and microbial lipases (Pantaleone *et al.*, 1992). On the other hand, Solovyeva *et al.* (1994) showed that chitosan sulfate (90,000 Da, degree of sulfation 1.3) is an activator of lipoprotein lipase; the observed decreased blood plasma VLDL and increased HDL levels, in agreement with Okunevich *et al.* (1992). Moreover, chitosan could be an alternative substrate for lipase *in vivo*, leading to partial inhibition.

Le Houx and Grondin (1993) studied the consequences of chitosan administration on 3-hydroxy-3-methylglutaryl CoA reductase in rats fed a sterol diet. This enzyme is the key regulatory enzyme of cholesterogenesis and produces mevalonic acid, a precursor of cholesterol. The enzyme levels remained close to the normal value with a 7.5% chitosan formula, and plasma high density lipoprotein cholesterol did not decrease. On the other hand, the enzyme activity was overstimulated in the sterol + cholestyramine group, where livers were smaller and yellowish. By this means the advantages of chitosan over cholestyramine have been clearly focused, but the nexus between chitosan and the enzyme has not been elucidated. In any event, Hirano and Akiyama (1995) point out that orally and intravenously administered chitosans (degree of acetylation 0.25, oligomeric and polymeric forms) do not accelerate the restoring of normal serum cholesterol levels in high-serum-cholesterol rabbits when they are switched to the cholesterol-free diet, although orally administered chitosans depress absorption in the intestine;

therefore, the hypocholesterolemic action takes place mainly (or only) in the digestive system.

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