

Interaction between Chitosan and Oil under Stomach and Duodenal Digestive Chemical Conditions

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Chitosan, the N acetylated derivative of chitin, has an effect on the absorption of dietary lipids, but there is not enough scientific knowledge about the mechanism.

To study the interaction between chitosan and oil, the action of this biopolymer has been evaluated through an experimental model of the stomach and duodenum tract, although the enzymatic activity had not been evaluated.

We microscopically confirmed that chitosan in a hydrochloric acid medium (pH 1.0–2.0) emulsified lipids and the emulsion was a water in oil in water type (w/o/w). When the pH value and speed of agitation were increased to mirror the duodenum medium conditions under which lipids are absorbed, the emulsion capacity was better with an increased number of droplets and the emulsion continued as the w/o/w type. At pH 6.2, chitosan precipitated and lipids were entrapped in the formed flocculus. The binding oil was quantitatively determined, and we also demonstrate that a larger oil quantity induced less retention, while the chitosan characteristics had no influence.

These observations allow us to postulate that the interaction between chitosan and oil inhibited duodenal absorption and enhanced lipid excretion.

Key words: chitosan; dietary lipid; water in oil in water emulsion (w/o/w); interaction digestive tract

The management of obesity is a major challenge for both physicians and patients. Conventional treatment based on dietary restriction and behavior modification generally results in only limited success, with the majority of obese patients unable to maintain short-term weight losses.^{1–3} Therefore, there is a recognized need for safe and effective adjunctive pharmacotherapy.

The use of dietary supplements, especially those for weight control, is increasing. Advertising claims for some of these supplements may give consumers unrealistic expectations. Few weight loss supplements are clinically tested for efficacy, yet their proliferation

continues. Chitosan-based supplements are sold as “oil trappers and oil magnets”.

Chitin is an amino-polysaccharide containing β -(1,4)-linkages as is present in cellulose. Chitosan is the deacetylated product of chitin. Chitin and chitosan are found in the exoskeletons of arthropods (such as crabs and lobsters) and in the cell walls of most fungi. Mammalian digestive enzymes can digest neither chitin nor chitosan.⁴

Chitosan is primarily consumed as a supplement and is often used to prevent dietary fat absorption as a mean for controlling weight.^{5,6} It could be classified as functional fiber, but there have been no studies about chitosan behaviour in the gastrointestinal tract. Despite an abundance of scientific research on chitosan, its properties and ability to bind bile salts and lipids and the specific mechanisms by which chitosan accomplishes such binding are still uncertain. The pioneering studies conducted with experimental animals by Sugano *et al.*,^{7,8} Nagyvary *et al.*⁹ and Kobayashi *et al.*¹⁰ have shown that chitosan, when is used in rat diets at moderate concentrations of 4–5%, can significantly decrease the levels of serum and liver cholesterol and triglycerides. Several *in vitro* and *in vivo* studies have demonstrated that chitosan could bind dietary lipids and thus interfere with their absorption from the gastrointestinal tract.^{11–15} Numerous additional studies involving rats,^{16–21} rabbits, hens and broilers,^{22,23} broiler chickens,^{24–26} mice,²⁷ and several other animals have shown similar results. Most of these observations have also been confirmed in humans.^{28–32} While the hypocholesterolemic and lipid-lowering effects of chitosan have been demonstrated in variety of species, the molecular mechanism for its activity is not yet fully understood.

There are some previously proposed mechanisms for cholesterol reduction. Animal models have suggested that a strong ionic attraction between chitosan and bile acids might be responsible for the cholesterol-lowering effect demonstrated in these experiments.^{7–21}

Another hypothesis suggests that, due to chitosan

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Abbreviations: DD, deacetylation degree; w/o/w, water in oil in water emulsion

Table 1. Characteristics of the Four Chitosans Used in the Study

Chitosan characteristics	1	2	3	4
Moisture (g%)	7.2 ± 0.15	6.5 ± 0.20	68.4 ± 0.05	6.0 ± 0.10
Ash content (g%)	0.42 ± 0.015	0.48 ± 0.018	0.35 ± 0.005	0.55 ± 0.017
Deacetylation Degree	78	85	94	95
Viscosity (mPas)	38	30	21	3

Mean ± standard deviation (n = 3)

being a weak base with pKa value of 6.5, it is capable of ionically binding anions such as free fatty acids and bile acids.³³⁾ Several studies have suggested that, in the proximal small intestine with a gradually increasing pH, the positively charged amino groups of chitosan may attract negatively charged fatty acids and bile acids in mixed micelles. This would result in the entire micelle becoming attached to chitosan, or disruption of the micelle by removal of the bile acids or fatty acids. In this environment of progressively more neutral pH, bile acids would be bound to a greater degree than fatty acids, due to their greater degree of ionization in the neutral pH range. However, whether chitosan binds entire micelles or disrupts micelles, the outcome would be a decreased absorption of lipids from the small intestine, accompanied by enhanced excretion of lipids, bile salts, and cholesterol.^{12,13,21–34)}

Gades and Stern³⁵⁾ have tested the fat-trapping capacity of a chitosan product in 12 men and 12 women and concluded that the amount of trapped fat was clinically insignificant, so is necessary that extensive scientific investigation continues to broaden our understanding of the characteristics and applications of chitosan. Due to its unique properties, this biopolymer has a variety of effects on weight loss and the management of cholesterol. Although the exact mechanism by which chitosan binds fat is not yet fully understood, its influence on weight loss, cholesterol lowering, and associated health benefits are being documented. As our knowledge of chitosan has grown, so has our appreciation of the realistic value of this compound. The opportunities for additional research in this area are plentiful.

This work studies the interaction between chitosan and sunflower oil with a chemical experimental model of the human digestive tract (gastric and duodenal environment).

Materials and Methods

Chitosan. All reagents were of either analytical grade or the highest purity available. Chitin was isolated from shrimp (*Pleoticus mülleri*) waste in our laboratory (LIBAQ). The raw material was homogenized and triturated in industrial equipment (Westinghouse DA-SO6). The product was rinsed with water at room temperature, as required, in order to remove the organic materials. The cleaned residue was treated with 9% (w/w) NaOH (Lab Chem, Inc., Pittsburgh, PA, U.S.A.)

at 65 °C for 90 min, to remove the proteins, demineralized by treating with 10% (v/v) HCl (Merck, Buenos Aires, Argentina) at 20 °C for 15 min, washed with water at room temperature and then air dried.

Chitosan 1, 2, 3, and 4 with different characteristics were obtained from crustacean chitin. They were directly prepared by heterogeneous deacetylation at 136 °C with 50% (w/w) NaOH for different times (1,2,3 or 4 h). Their characteristics are shown in Table 1.

Viscosity was measured in a solution of 1% of each chitosan (w/v) in 1% (v/v) acetic acid with a Brookfield DV-IV+ viscometer (Brookfield, Stoughton, MA, U.S.A.) using spindle 21 run at 50 rpm.

Digestive chemical experimental model. The digestive chemical experimental model enabled mimicking, in the laboratory, the *in vivo* reactions that take place in the stomach and duodenum. The experiments were based on the following approaches: 1 g of chitosan 3 (the recommended dose for humans)³⁶⁾ was stirred in 400 ml of 0.1 M HCl (Merck) for 1 h at pH 1.0–2.0, 30 rpm and 37 °C to reproduce the gastric environment. Different amounts of sunflower oil (a commercial type purchased in a local supermarket) were then added while stirring for 30 min at the same speed and temperature. The amounts added were related to the different lipid contents in the human diet (4.0 g corresponding to 12% of lipid intake, 10.0 g corresponding to 30% of lipid intake, 16.0 g corresponding to 48% of lipid intake and 32.0 g corresponding to 96% of lipid intake). The emulsions formed were taken from an acidic medium to pH 6.8 with 15 g/l of NaHCO₃ (Sigma Chemical Co., St. Louis, MO, U.S.A.). The stirring speed was increased from 30 to 300 rpm and the temperature maintained at 37 °C to reproduce the duodenal environment.

Influence of chitosan characteristics. The digestive chemical experimental model was operated with different chitosans (1,2,3 or 4, shown in Table 1) and 10.0 g of sunflower oil to evaluate the influence of the chitosan characteristics.

Quantification of entrapped oil. The entrapped and absorbed oil in the gel formed at pH 6.8 was released by a solvent treatment and quantified by the gravimetric method. The flocculus was separated by filtration and washed with 30.0 ml of ethyl ether to separate the absorbed oil, this flocculus was stirred 3 times with 30.0 ml of ethyl ether each time to extract the entrapped

oil. The solvent was eliminated, and the entrapped oil was gravimetrically determined.

Microscopic study. Samples of each emulsion were placed between slides, observed with an Olympus BH-2-UMA microscope and then photographed with a Sony CCD IRIS/RGB camera to study the droplets characteristics.

Statistical analysis. The characteristics of each chitosan (Table 1) and the relationship between the amount and percentage of entrapped oil (Fig. 6) are expressed in each case as the mean \pm SE for *n* replicates. Spearman's rank correlation (non-parametric data) was used to test the direction and strength of the relationship between the entrapped oil and different sunflower oil amounts. The influence of different chitosan characteristics on the percentage of entrapped oil was statistically analysed by a one-way analysis of variance (ANOVA) ($p > 0.90$).

Results and Discussion

We used in this study chitosans with different characteristics (Table 1) obtained under different experimental conditions.

Digestive mimicking study

We have demonstrated in previous works^{37–40} that chitosan in acetic and hydrochloric acids stabilized an emulsion of the w/o/w type.

The interaction between chitosan and sunflower oil (as the lipid component of the diet) was studied by an experimental model that reproduced *in vitro* the gastrointestinal tract conditions, particularly the gastric and duodenal environment.

The hydrochloric acid concentration, volume, temperature and the stirring speed were selected according to the *in vivo* conditions. Sunflower oil was used because it is the most commonly consumed in Argentina. The oil quantities employed in this study were related with the different lipid contents of the human diet.

The digestive experimental model allowed us to confirm that chitosan emulsified sunflower oil when it was stirred at a low speed in an HCl medium of pH 1.0–2.0 (the experimental gastric environment). The emulsion was a w/o/w type and microscopically few emulsified oil drops were observed. (Fig. 1)

The experimental model for the duodenal environment enabled the pH value and stirring speed to be increased. When the pH value was increased to 5.8 by adding sodium bicarbonate and with a stirring speed of 300 rpm, the chitosan emulsion-forming capacity was improved. We observed microscopically that the emulsion continued to be of the w/o/w type, but with a greater number of emulsified oil droplets (Fig. 2).

Finally taking into account the pK value of chitosan ($\cong 6.2$), this biopolymer was precipitated at the duodenal pH value, and the entrapped lipids in the formed

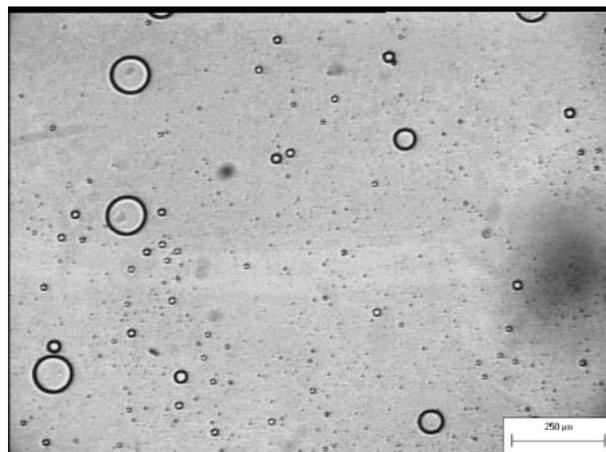


Fig. 1. w/o/w Type of Emulsion of Sunflower Oil Stabilized with Chitosan in an Experimental Gastric Environment (pH 1.0–2.0).

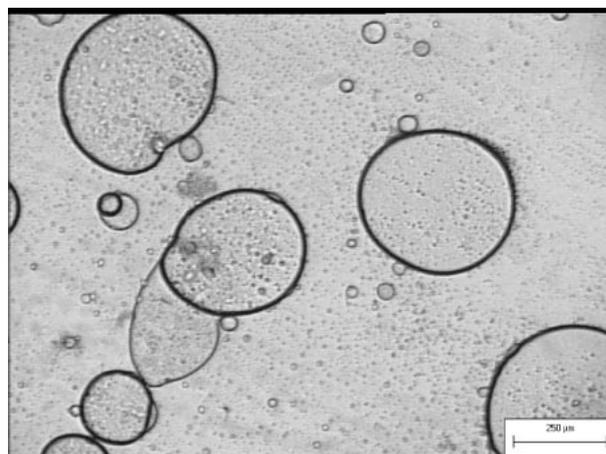


Fig. 2. w/o/w Type of Emulsion of Sunflower Oil Stabilized with Chitosan in an Experimental Duodenal Environment (pH 5.8).

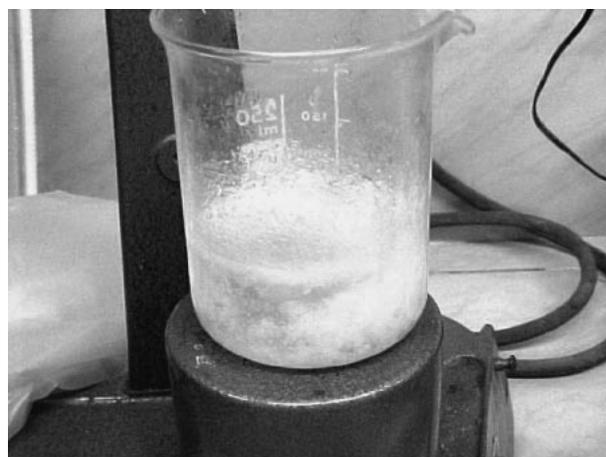


Fig. 3. Chitosan Flocculus Formed in the Experimental Duodenal Environment (pH 6.5–7.5).

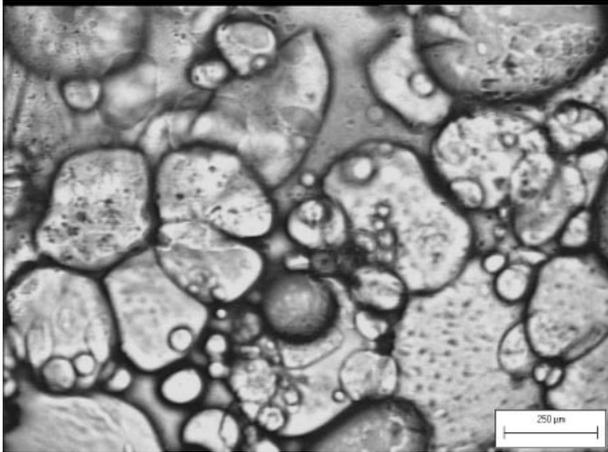


Fig. 4. Photomicrograph of the Chitosan Flocculus in the Experimental Duodenal Environment (pH 6.5–7.5).



Fig. 5. Isolated Chitosan Flocculus.

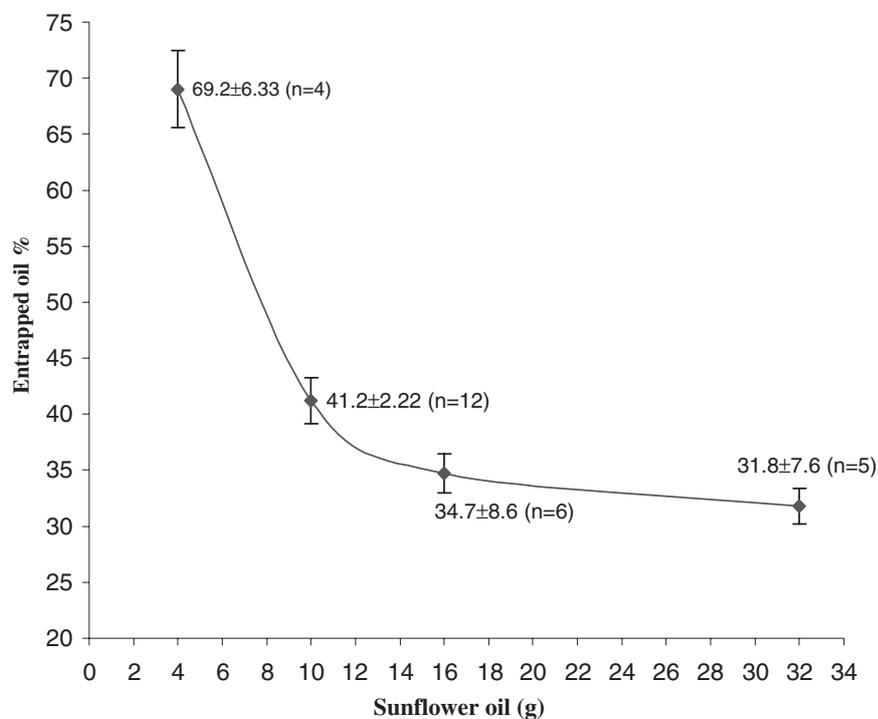


Fig. 6. Relationship between Entrapped Oil and Lipid Intake.

flocculus and the medium viscosity were decreased (Fig. 3).

A photomicrograph and macroscopic view of the isolated flocculus are respectively shown in Figs. 4 and 5.

These observations allow us to postulate that the interaction between chitosan and oil in the digestive tract involves transformation of the stomach emulsion to an intestinal flocculus of precipitated chitosan. The entrapped oil cannot be absorbed through the intestinal wall. This information can be used to design or select new polysaccharides with optimum lipid-reducing characteristics for incorporation into functional foods.

Entrapped oil study

It is important to point out that the dosages of chitosan used in this study are equivalent to the recommended doses for humans, and the oil quantities employed are equivalent to 12, 30, 48 and 96% of the dietary energy intake. The percentage of entrapped oil for the different oil quantities used are shown in Fig. 6. Spearman's rank correlation value is -1 for these results, being a perfect negative correlation.

These results suggest that ingesting chitosan can effectively reduce lipid absorption and thus cause weight loss. We also observed that when the dietary oil content was increase, the entrapped oil percentage decreased.

Table 2. Oil Entrapped by the Four Types of Chitosan

Chitosan (1.0 g)	Oil (g)	Entrapped oil (g)	Trapped oil %
1	9.5	3.5	37
1	10.0	4.1	41
1	9.9	4.2	43
2	10.0	4.2	42
2	9.8	3.7	38
2	10.5	4.5	43
3	10.1	4.5	45
3	9.9	4.0	41
3	10.2	3.9	39
4	10.3	4.3	42
4	11.5	4.6	40
4	12.0	5.1	43

1-DD = 85% η = 12 cP 2-DD = 78% η = 38 cP

3-DD = 95% η = 3 cP 4-DD = 94% η = 21 cP

We also studied the influence of chitosan characteristics on the interaction between the biopolymer and oil. We simulated the chemical conditions in the digestive track and worked with four different chitosans with 10.0 g of sunflower oil corresponding to 30% of the energy content in the diet. The results obtained are shown in Table 2.

We observed that the entrapped oil percentage was $41.16 \pm 2.22\%$ ($n = 12$), and an analysis of variance (ANOVA) at $p < 0.1$, disclosed no significant differences in chitosan behavior according to its characteristics.

Conclusion

The results of this study allow us to propose that chitosan is dissolved in an acidic medium like that found under gastric condition and emulsifies oil, before forming a flocculus under the higher duodenal pH value. The flocculus formed entraps dietary oil and prevents lipid absorption through the intestinal wall, so the oil is excreted with the feces. This mechanism for the action of chitosan can be assumed to reduce weight. The results were better with a diet involving a low lipid content and were independent of the chitosan characteristics.

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