Influence of Chitosan on Lipase Activity and Bioavailability of Fatty Acids During Lipid Digestion.

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Chitosan is a cationic biopolymer used increasingly to reduce fat absorption. However, current attempts to explain the mechanism of action have been a subject of controversy. The objective of this study was to investigate how different chitosans influenced triacylglyserol (TAG) digestion and fatty acid (**FFA**) absorption. A stock oil-in-water emulsion was prepared by homogenizing corn oil with 10mM SDS and 1% Tween 20 surfactant solution at pH 3. The emulsion was mixed with 0.025-0.1wt% HCl-dispersed chitosans (**LMW**: MW~300kDa, DDA~90%; **HMW**: MW~1200kDa, DDA~90% and **LDDA**: MW~1200kDa, DDA~40%)) at pH 3 ($c_{oil,total}$=3wt%). The pH was raised to 5 where 10mmol bile salts and 8mg/ml lipase where added along with NaOH increasing the pH to 7. Solution was incubated in a shaking incubator (150 rpm) for 2 hours at 37°C. To investigate **FFA** binding the solution was filtered and the reaction stopped. Then the **FFA** released where extracted into 3 part hexane and 2 part isopropanol solvent witch was then evaporated and re-dissolved in acetone. Finally the acetone/FA solution was titrated with 0.01M NaOH. Total lipase activity was not inhibited but rather increased for all chitosan at 0.025-0.1% chitosan concentration except for **HMW** chitosan witch showed similar activity as blank. All chitosans seemed to inhibited lipase activity at low chitosan concentration 0.025% except for **LDDA** witch only seemed to increase activity. Chitosan seemed to bind **FFA** at pH 7 rather strongly the most effective chitosan was LMW chitosan witch bound 54.93% ±0.38% compared to blank witch had 0.60% ±0.30% in the filter. **FFA** bound to chitosan changed significantly with chitosan concentration (p=0.001). Our results indicate that chitosan increased lipase activity but inhibited FA absorption in the small intestine. This contradicts most of the research on chitosans influence on bioavailability of fat and calls for changed emphases in the subsequent investigations.

Introduction

Our working theory suggests that chitosan forms a "screen" around the oil droplets [1] [2] [1] because the droplets are negatively charged and the chitosan is positively charged at pH 2, witch is the normal pH in the stomach. When this emulsion reaches the *duodenum* the pH increases, at pH 7 the chitosan precipitates and chitosan-oil-droplets starts to flocculate out of the solution forming a gel entrapping the oil droplets [3]. But before the chitosan precipitates the lipase is secreted in the proximal part of the duodenum the pH is increasing from 2 to 5 within the proximal 10 cm of the duodenum [4]. And therefore the lipase has plenty of time to digest the fat before the chitosan secondary emulsion precipitates. Researches inspecting the influence of bile salts on chitosan has suggested that bile salts will precipitate chitosan at any pH from 3-6 [5]. Therefore the bile salts that are under normal circumstances supposed to increase the digestibility of the dietary fat now will

flock out the chitosan secondary emulsion resulting in formation of solid gel structure. This is expected to decrease the bioavailability of the dietary fat.

Another theory states that the chitosan does not influence the pancreatic lipase digestion or at least not much but rather influences the absorption of FFA by binding FFA and bile salts. Usually people discard this theory right away because at the pH in the small intestine the chitosan will not be charged. This is really a misunderstanding of one of the laws of chemistry i.e. the Henderson-Hassalbach equation (equation 1) which states that charged groups with pk_a of 6.5 will still have charges at pH 7 and even higher like stated above. Therefore it is not unlikely that chitosan will be able to bind FFA at pH similar to the pH in the small intestine.

The complexity of the suggested mechanisms involved in the gut function of chitosan is obvious and might conceal details which could contribute to explaining inconsistent findings in many of the clinical investigations on the influence of chitosan on weight reduction or fat digestibility. Among such details to be considered are binding of chitosan to bile salts obstructing their extremely important role in fat digestion [6] [7], [8] [9]. One interesting theory is that chitosan forms a sheet around the oil droplets and when lipase starts forming f.a. chitosan binds the f.a. which would then form a chitosan-f.a. sheet around the oil droplets and inhibit lipase directly since f.a. can inhibit lipase activity [10]. One the other hand this could also have the reverse effect because the concentration of the f.a. in the solution should decrease.

The digestive system is a very complex system involving many parameters that can influence the activity of chitosan, such as pH, ratio of different types of bile, phospholipids, amount of enzyme etc. This suggests that in order to get reliable results *in vitro* experiments have to involve careful simulations of the digestive system which are designed to be as true to the *in vivo* systems as possible followed by complimentary *in vivo* trials employing experimental animal models and humans.

Purpose of this project is to construct models which simulates specific parts of the digestive system, aimed to enhance our understanding of the mechanisms responsible for the functionality of dietary chitosan in the human GI tract. It is our hope that these results will help to improve the design of subsequent *in vivo* experiments, aimed to reveal the gut function of chitosan and its contribution to weight management in humans. We also believe that our results will help to optimize polymer composition for optimum GI functionality.

Materials and methods

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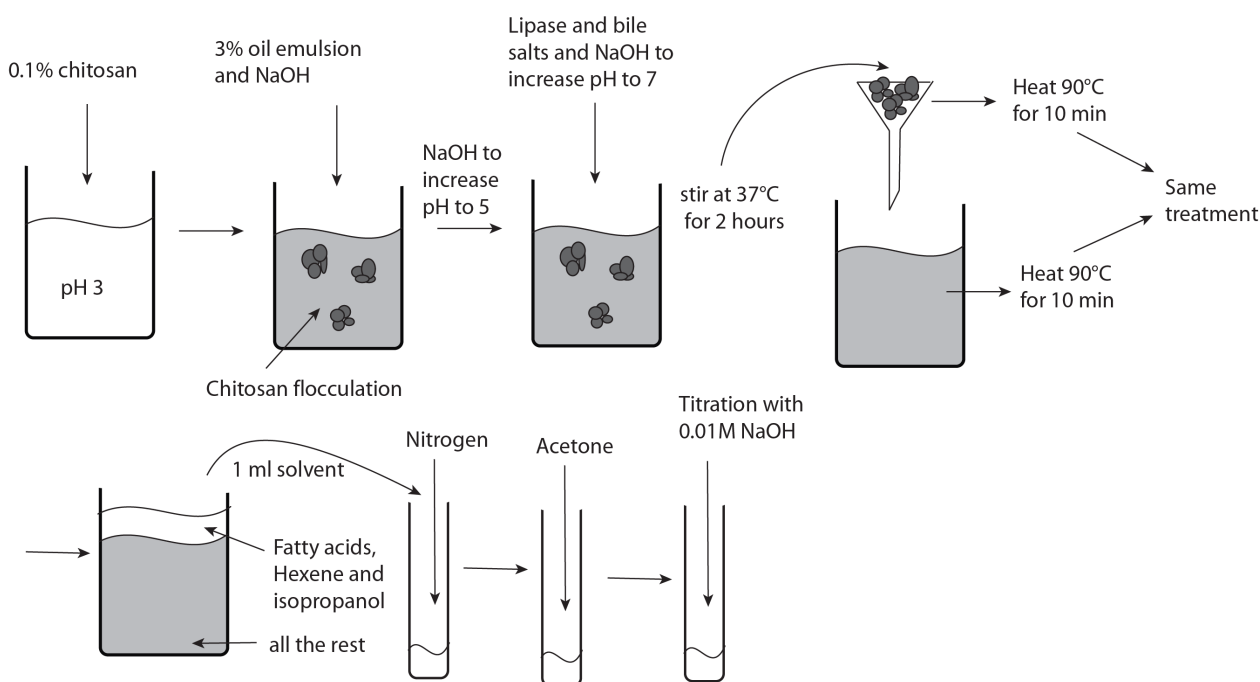
Results and discussion

The results herein showed can be applied to increase our understanding on the effect of chitosan on emulsion and FFA under conditions similar to the digestion system. Furthermore these results can be applied in the formation of a stable emulsion with chitosan. These results can there fore have implication on both food industry and the pharmaceutical industry.

Lipase activity in the blank

Lipase activity measurements revealed unexpected results (Figure 11). First of all under the conditions used only 60% activity was observed inticating that the lipase only digested 60% of the oil. A closer look at the pancreatic lipase mechanism may explain this. Since pancreatic lipase usually does not digest FA's at the sn-2 position witch is the fatty acid in the middle of the glycerol backbone [11] witch is normally absorbed before it is digested because digestion of the sn-2 position is usually slower than the sn-1 and sn-3 positions [12] [13]. Also since simulation of the absorption of FFA trough the unstirred water layer was not faceable under the experimental conditions which would have been a simulation of what normally takes place in the digestion system we are faced with accumulation of FFA. The accumulation of FFA in the solution was a problem since it has been postulated that high concentration of FFA will inhibit pancreatic lipase activity [10]. This inhibition is also known in Gastric lipase [14]. Other researchers have showed activity inhibition at high oil concentration [15].
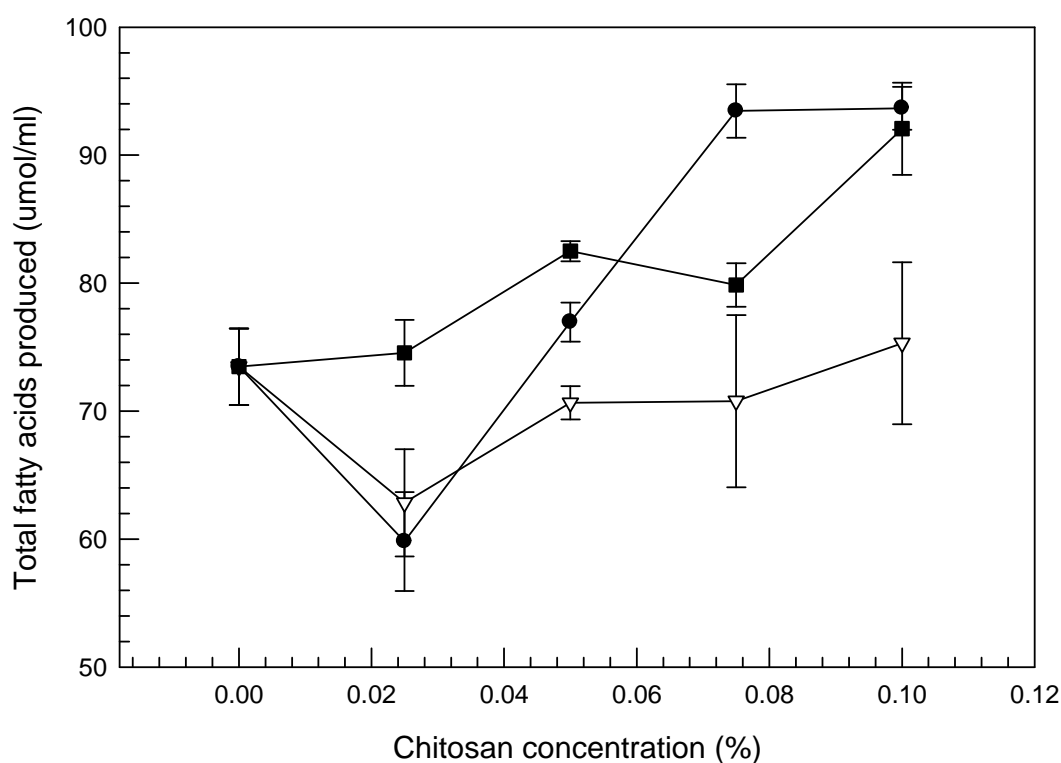

Figure 11. Influence of chitosan concentration on lipase activity. Three chitosans were studied:

LMW-90% = 200 kDa, 90% DDA (●); HMW-90% = 750 kDa, 90% DDA (Δ); HMW-40% = 750

kDa, 40% DDA (■).

Influence of chitosan flocculation on lipase activity

The strength of the gel formation and its possible fate during digestion was of interest where the gel formation with and without chitosan was examined. Before addition of pancreatic lipase there were

extensive flocculated structures then after addition of lipase the gel started to break apart and after 2 hours free oil and chitosan fibers remained (Figure 12). This indicates that formation of gel structure is not likely to influence the lipase activity since the gel structure is broken down under condition simulating the digestive system.
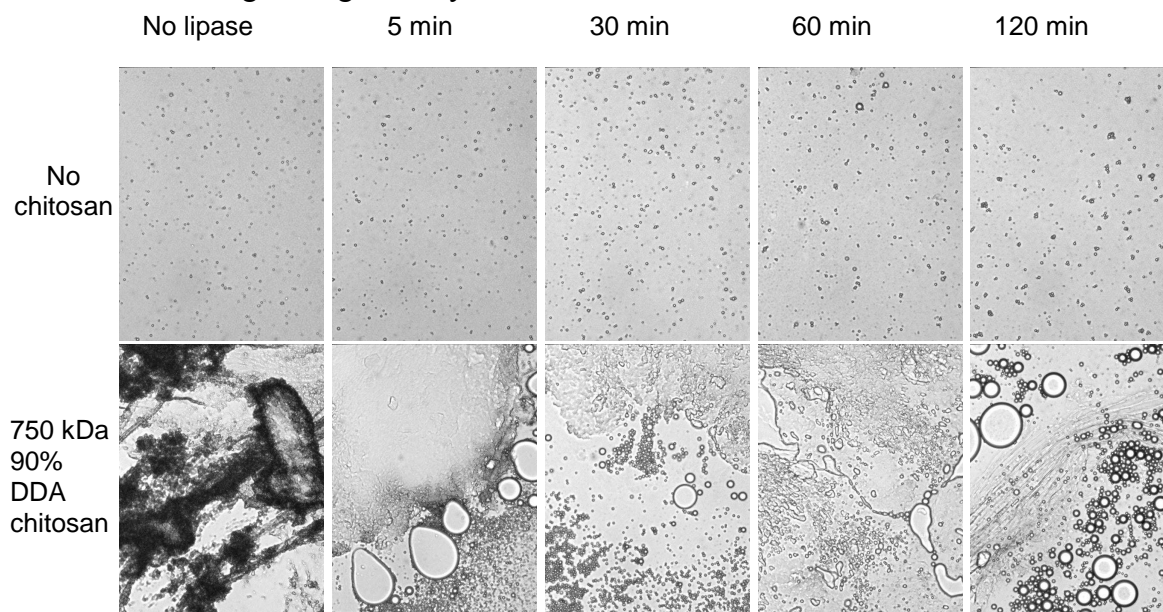

Figure 12. Microstructure of chitosan-oil emulsion aggregates at pH 7.

FFA binding

Since first lipase experiment indicated that chitosan bound FFA that mechanism was tested further. Given that chitosan will flocculate out when bile salts are added and at pH 7 it is possible to filter the flocculation out. Therefore it is possible to measure the amount of FFA in both the chitosan precipitation and the filtrate. The results supported the theory that chitosan is able to bind FFA at pH 7 (Figure 13). A closer look at these results reveals a similar pattern as in figure 11. LMW and LDDA chitosan both show increasing FFA binding with increasing chitosan concentration (Figure 13) and HMW chitosan also increased FFA binding with increasing chitosan concentration but the FFA binding was lower. These results fit to the theory because if the chitosan can bind more FFA the lipase can digest more TAG before it is inhibited by high concentration of FFA. Therefore LMW chitosan increases the lipase activity most at highest concentration since it is able to bind the highest amount of FFA at high concentrations.

Even tough these results do not directly prove that chitosan was able to inhibit FFA absorption they indicate that that might be the case. By binding FFA chitosan will probably be able to keep the FFA from going trough the unstirred water layer witch separates the mucosal cells from the small intestine content. The only possibility for FFA that are bound to the chitosan polymer to be absorbed is that they are released so they can form micelles witch then can diffuse trough the unstirred water layer [12]. Formation of micelles is strongly dependent on availability of bile salts witch help micelle formation and increase its water solubility [16]. Since chitosan can bind bile salts [5] they can not play their role in forming micelles and therefore FFA diffusion trough the unstirred water layer will probably be further reduced.
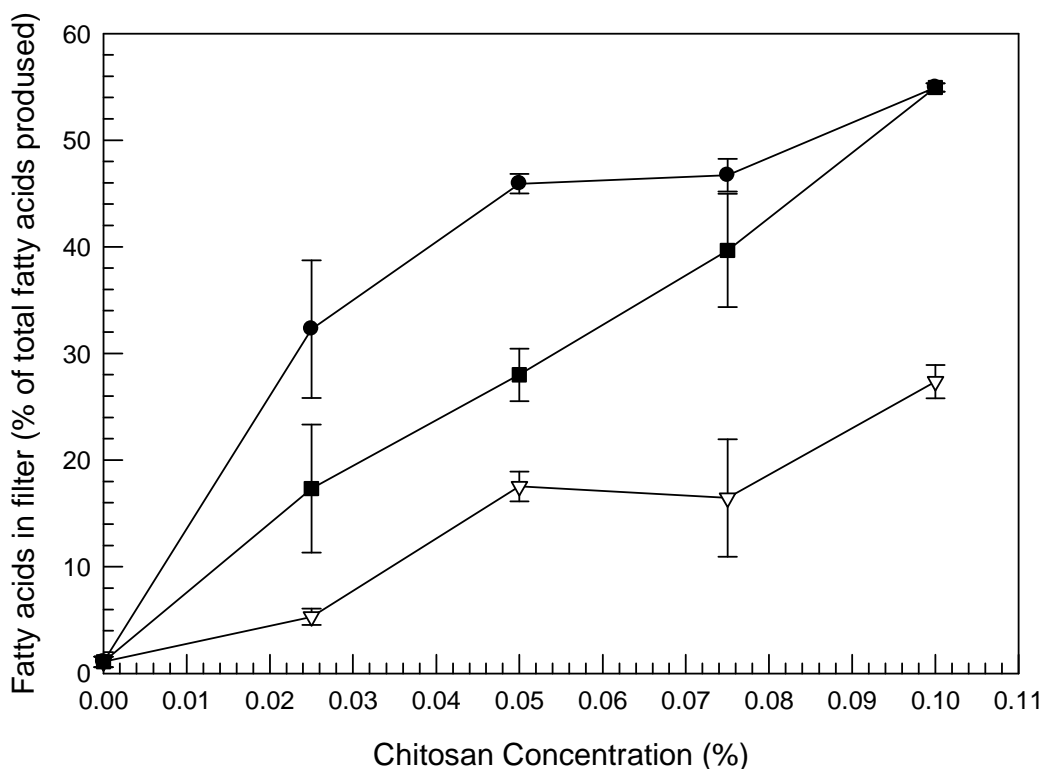
Figure 13. Influence of chitosan concentration on fatty acid binding. Three chitosans were studied: LMW-90% = 200 kDa, 90% DDA (●); HMW-90% = 750 kDa, 90% DDA (Δ); HMW-40% = 750 kDa, 40% DDA (■).

CONCLUSIONS

The results suggest that the mechanism of the action of chitosan in the digestive tract is even more complex than previously suggested. Chitosan can cause a vide variety of both stabilization and destabilization of oil emulsions depending on a wide variety of factors such as pH, emulsion electrostatics, emulsion particle size, chitosan concentration, chitosan characteristics, surface active materials such as SDS and bile salts, in what order the solution is mixed and other extrinsic and intrinsic factors. Given the complexity of the mechanism it is not surprising that argumentation on how and if chitosan can affect the bioavailability of dietary fat exists. After two years of research we finally have reached the conclusion that chitosan is not likely to inhibit lipase digestion under condition close to digestion condition. On the other hand chitosan was found to exhibit some FFA binding effects thus decreasing absorption of FFA in the digestion.

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