

## Evaluation of the effect of chitosan oleogel structuring on the release of fatty acids during the in vitro digestion process

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### Introduction

The consumption of processed foods is associated with the development and exacerbation of non-communicable chronic diseases, partly attributed to the presence of saturated fatty acids and the extensive utilization of interesterified fats [1]. Chitosan-based oleogel represents a promising alternative for the reduction of saturated fatty acid levels in processed foods. Nevertheless, this system may also demonstrate potential biological activity due to its capacity to enhance the content of unsaturated fatty acids in food products and serve as a controlled release carrier for lipids [2]. The controlled release capacity of the oleogel is associated with its technological characteristics, such as hardness and oil loss, since the network structure, characterized by these parameters, can impact the effect of intestinal lipase, either increasing or reducing the final release of fatty acids [3]. However, these properties of chitosan oleogel have not yet been thoroughly investigated. Therefore, the aim of this study was to investigate the role of chitosan-structured oleogel as a controlled lipid release vehicle during the in vitro digestion process.

### Material and Methods

Chitosan-based oleogels using soybean oil were prepared by the emulsion-template method [4]. The oleogels were structured with different concentrations of chitosan (0.42, 0.75, and 1.075%) and vanillin (1.0% w/w), a crosslinking agent. Liquid oil was used as a control.

In vitro digestion followed INFOGEST 2.0 protocol, adapted for high fat samples [5]. Liquid oil and oleogels underwent the following stages: Oral stage: This stage involved a simulated oral phase fluid and the sample (1:1 w/w), maintained under agitation at 37°C for 2 minutes. Gastric stage: The oral stage was acidified to pH 3.0 with HCl, followed by the addition of CaCl<sub>2</sub> (0.15 mM) and simulated gastric fluid to the bolus formed in the previous stage (1:1 v/v). Solutions of pepsin (2000 U/mL) and gastric lipase (60 U/mL) enzymes were added, and the stage was maintained under agitation at 37°C for 2 hours. Intestinal stage: The gastric stage was alkalized to pH 7.0 with NaOH, followed by the addition of CaCl<sub>2</sub> (0.6 mM) and simulated intestinal fluid to the chyme formed in the previous stage (1:1 v/v). Solutions of pancreatin (100 U/mL) enzymes were added, and due to the lipid-rich nature of the substance, a lipase solution (2000 U/mL) was included. Finally, a prepared bile extract (10 mM) was added. The intestinal stage was maintained under agitation at 37°C for 2 hours.

The assessment of the % release of fatty acids and their release rate was conducted by analyzing various time points during intestinal digestion (2.5, 5, 10, 15, 30, 60, 90, and 120 minutes). Free fatty acids will be determined through acid-base titration, and the rate of lipolysis will be calculated [6].

### Results and Discussion

The total percentage of fatty acids released at the end of the intestinal stage of in vitro digestion varied according to the concentration of chitosan used in the structuring of the oleogel. Specifically, the liquid oil exhibited  $78.4 \pm 1.3\%$  of free fatty acids, while oleogels showed values that ranged from approximately  $54.7 \pm 0.9\%$  (oleogels with 0.42% and 0.75% chitosan) to  $49.9 \pm 2.8\%$  (oleogels with 1.075% chitosan). This reduction in the release of fatty acids may be associated with the structure of the polymer network formed by the interaction of chitosan with vanillin, which likely acts as a physical barrier, diminishing the effect of pancreatic lipase during digestion [7].

In addition to the effect on the reduction of the final release of free fatty acids, when evaluating the release kinetics, considering the release of fatty acids over time, it was observed that the oleogel developed with the highest concentration of chitosan led to controlled release of the fatty acids compared to the other oleogels and the liquid oil. This was evidenced by a change in the rate constant ( $k$ ) of  $k=0.0354$  (1.075% chitosan oleogel,  $R^2=0.9954$ ),  $k=0.0197$  (0.75% chitosan oleogel,  $R^2=0.9528$ ),  $k=0.0184$  (liquid oil,  $R^2=0.967$ ), and  $k=0.2453$  (0.42% chitosan oleogel,  $R^2=0.8959$ ). In other words, the increase in chitosan concentration resulted in a deceleration of the fatty acid release over the course of intestinal digestion. The structuring of oil to create oleogels using various structuring agents from different categories, such as polymers (ethyl cellulose or chitosan), proteins (whey protein), or waxes (beeswax, rice bran wax), also demonstrated control over fatty acid release, which can be attributed to the reduction in the effect of lipase activity, caused by the formation of a physical barrier within the oleogel structure [6, 8].

## Conclusion

The structuring of soybean oil with chitosan led to a reduction in the total release of fatty acids during in vitro digestion, regardless of the chitosan concentration used. Furthermore, the oleogel structured with the highest chitosan concentration exhibited controlled release of fatty acids during the intestinal digestion phase, suggesting that in addition to the reduction in the total release of fatty acids, there was also a deceleration in their release kinetics over time.

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