# Evaluation of Chitosan-Based Oleogels with Different Oil Phases on Free Fatty Acid and Carotenoid Bioaccessibility After In Vitro Digestion

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## Introduction

The consumption of processed foods is associated with the development of non-communicable chronic diseases, partly attributed to the presence of saturated fatty acids and the extensive utilization of interesterified fats [1]. Chitosan-based oleogels represent a promising alternative for the reduction of saturated fatty acid levels in processed foods. This system may exhibit potential biological activity due to its ability to enhance the content of unsaturated fatty acids in food products and may serve as a carrier for lipophilic bioactive compounds, such as carotenoids [2, 3]. The controlled release capacity of oleogels is linked to their three-dimensional network structure that can impact the activity of intestinal lipase, modulating the final release of fatty acids and the bioaccessibility of carotenoids [3]. However, these properties of chitosan-based oleogels have not yet been thoroughly investigated, especially when distinct oil phases are used to obtain these oleogels. Therefore, the aim of this study was to investigate the role of chitosan-based oleogel made with different oil phases and chitosan concentrations in reduction of free fatty acid release and its impact on carotenoids bioaccessibility after the in vitro digestion process.

## Material and Methods

Chitosan-based oleogels using soybean, *dendê*, and annatto seed oils were prepared by the emulsion-template method [4]. The oleogels were structured with different concentrations of chitosan (0.4% and 1.0%) and vanillin (1.0% w/w), a crosslinking agent. Liquid oils were used as a control.

In vitro digestion followed INFOGEST 2.0 protocol, adapted for high fat samples [5]. Liquid oils 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 CaCl2 (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 CaCl2 (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 total free fatty acids released and carotenoids (α- and β-carotene in *dendê* oil and bixin in annatto seed oil) were conducted by analyzing the endpoint of intestinal digestion. Free fatty acids were determined through acid-base titration, and carotenoid identification and quantification were performed by HPLC-DAD. Their bioaccessibility was calculated [6].

## Results and Discussion

The total percentage of fatty acids released at the endpoint of intestinal digestion varied according to the concentration of chitosan used in the oleogel. Regardless of the oil phase, liquid oil exhibited a higher release of free fatty acids, with values around 76%. In contrast, oleogels showed lower percentages, ranging from approximately 54% for oleogels containing 0.4% chitosan to 49% for those with 1.0% chitosan. No significant statistical differences were observed between the different oil phases. This reduction in fatty acid release 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, reducing the effect of pancreatic lipase during digestion [7]. Therefore, the type of oil phase did not impact free fatty acid release, whereas the chitosan content did.

The bioaccessibility of carotenes and bixin generally depends on the rate of lipolysis, as they require micelle formation during digestion to be available for absorption. Structuring oil phases with chitosan hindered this bioaccessibility, with very low values (up to 0.7%) observed for oleogels with 0.4% chitosan and no detectable amounts for oleogels with 1.0% chitosan. Previous studies have reported the formation of multilayer emulsions stabilized by chitosan and soy protein isolate also reduced the bioaccessibility of total carotenoids. It is suggested that the incomplete digestion of emulsions by lipases could leave some carotenoids trapped in the non-digested oil droplets, affecting their absorption. Additionally, carotenoids may become entrapped within large chitosan aggregates formed due to the alkaline conditions during duodenal digestion. Considering the reduction in lipid digestion, the bioaccessibility of carotenoids in chitosan-based oleogels may also be limited by their entrapment in the chitosan structure. The different structuring agents used to create oleogels, such as polymers (ethyl cellulose or chitosan), proteins (whey protein), or waxes (beeswax, rice bran wax), have also demonstrated a reduction in free fatty acid release. However, there are controversies regarding their effect on carotenoid release. These effects may be attributed to the reduced lipase activity caused by the formation of a physical barrier within the oleogel structure, as well as the nature of the structuring agent, which could either hinder or facilitate carotenoid digestion [6, 7].

## Conclusion

Chitosan-based oleogels reduced the total release of free fatty acids after *in vitro* digestion, regardless of oil phase and the chitosan concentration. However, these oleogels also reduced the bioacessibility of the carotenoids evaluated in this study (α- and β-carotene, and bixin). Therefore, chitosan-based oleogels can be used to produce low-calorie food products with low caloric value, but they are not effective as carriers for carotenoids.

**Acknowledgments**

The authors would like to express appreciation for the support of the sponsors FAPERJ (E-26/210.915/2021) and CAPES (Finance Code 001).

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