Carotenoid-Microbiota Dynamics: *In Vitro* Analysis of Gut Modulation and Associated Health Benefits

Helena R. Rocha[1], Rui C. Morais[1], Manuela E. Pintado[1], Ana M. Gomes[1] and Marta C. Coelho[1]

[1] CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal
H.R.R.: mhrocha@ucp.pt; R.C.M.: ; M.E.P.: mpintado@ucp.pt; M.C.C. - Corresponding author: mcoelho@ucp.pt

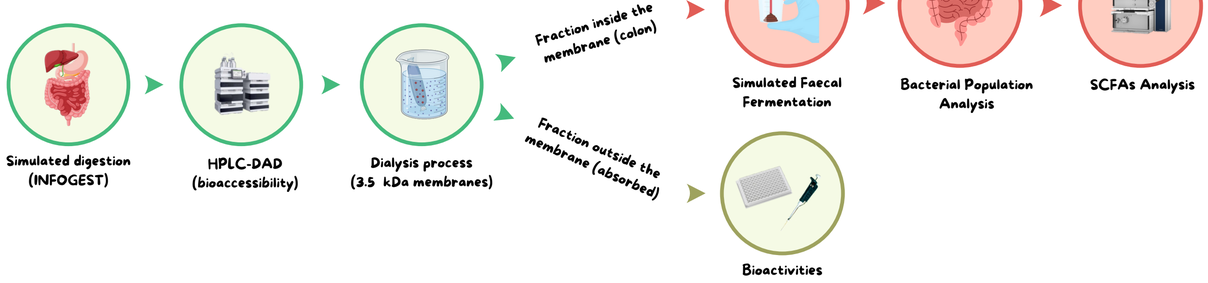
* Corresponding author: mcoelho@ucp.pt

INTRODUCTION

Carotenoids, **natural lipid-soluble pigments** abundant in various fruits and vegetables, play a significant role in our diet by adding vibrant colours to our meals (1). Humans and animals cannot synthesize these compounds, emphasizing the importance of dietary intake for reaping their benefits (2,3). Besides enhancing the visual appeal of food, carotenoids are renowned for their **health advantages**, serving as potent antioxidants and supporting eye health and immune function (3). However, realizing the full potential of carotenoids for human health faces challenges, primarily centered around their **bioaccessibility** (4,5). The journey of carotenoids from ingestion to beneficial impact is filled with obstacles, with digestion presenting a significant challenge. The chemical composition of carotenoids encounters resistance and degradation within protein complexes and the intricate structures of plant cell walls during digestion. Various factors such as dietary sources, food composition, matrix structure, lipid presence, and interactions with other compounds further complicate the bioaccessibility of carotenoids (6).

Noncommunicable diseases (NCDs), responsible for 41 million deaths each year, are often associated with unhealthy dietary habits. To combat this, nutrition and health organizations recommend a diet rich in fruits and vegetables (5). These foods are abundant in carotenoids, lipid-soluble phytochemicals known for their **health-enhancing properties**, including antioxidant, anti-diabetic, and anti-mutagenic effects (3,7). The **intestinal microbiota** (IM) significantly influences the efficiency of carotenoids (8). The IM plays a vital role in the absorption and metabolism of carotenoids, as a balanced diet can modulate the composition of the IM, promoting the growth of beneficial microbes and inhibiting harmful ones. Additionally, the IM synthesizes and releases various **metabolites**, which can be absorbed into the circulatory system, influencing the host's health (9). These interactions are crucial for understanding carotenoids' preventive and therapeutic potential.

METHODOLOGY



OBJECTIVE

This study aimed to investigate the **interaction between carotenoids and the intestinal microbiota** during simulated gastrointestinal digestion and absorption. It examined three specific carotenoids - beta-carotene, lutein, and lycopene - alongside a pigment mixture (MIX) and the alga *Osmundea pinnatifida*. The research focused on how these carotenoids influence **bioaccessibility**, **absorption**, **microbial dynamics**, and **organic acid production**. The study evaluated carotenoids' **antioxidant**, **antidiabetic**, and **antimutagenic** properties, offering insights into their potential health benefits.

RESULTS

Table 1. Carotenoid profile for the tested conditions at each simulated GIT phase, with their respective concentrations (Mean ± standard deviation (SD)) in mg/L. SSP - simulated salivary phase; SGP - simulated gastric phase; SIP - simulated intestinal phase; IM - dialysis phase inside the membrane; OM - dialysis phase outside the membrane; NI - not identified.

	BETA-CAROTENE		LUTEIN	
GIT PHASE	CAROTENOID	MEAN ± SD (mg/L)	CAROTENOID	MEAN ± SD (mg/L)
SSP	BETA-CRYPTOXANTHIN	3x10-3 ± 9x10-5	LUTEIN	3x10-2 ± 3x10-7
	LYCOPENE	3x10-2 ± 9x10-4	NI	----
SGP	BETA-CRYPTOXANTHIN	9x10-4 ± 6x10-5	LUTEIN	3X10-2 ± 3X10-7
	LYCOPENE	5X10-3 ± 6X10-5	NI	----
SIP	BETA-CRYPTOXANTHIN	8X10-4 ± 2X10-4	LUTEIN	1X10-1 ± 4X10-5
IM	BETA-CRYPTOXANTHIN	5X10-4 ± 2X10-5	NI	----
	BETA-CAROTENE	1X10-1 ± 1X10-4	NI	----
OM	NI	----	NI	----

Table 2. Recovery indexes (%) for the carotenoids plain β-carotene and plain lutein at the GIT sampling phases.

CAROTENOID GROUP	GIT PHASE	RECOVERY INDEX (%)
BETA-CAROTENE	IM	0.4
LUTEIN	SSP	0.02
	SGP	0.04
	SIP	0.27

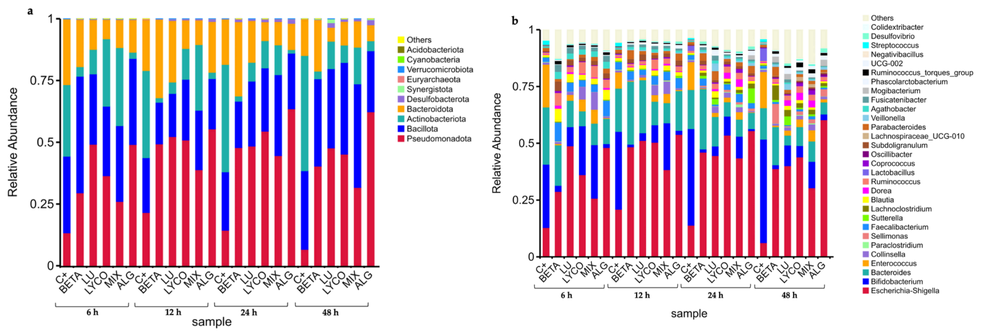


Figure 1. Phyla (a) and genera (b) relative taxonomic abundances from each tested condition at each time-point. (C+: control; BETA: β-carotene; LU: lutein; LYCO: lycopene; MIX: mixed solution of β-carotene, lutein, and lycopene; ALG: *Osmundea pinnatifida*).

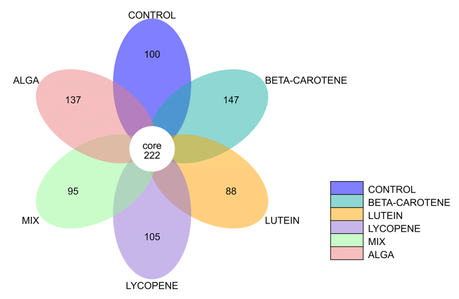


Figure 2. Flower diagram of each sample group. MIX: mixed solution of β-carotene, lutein, and lycopene. Alga: *O. pinnatifida*.

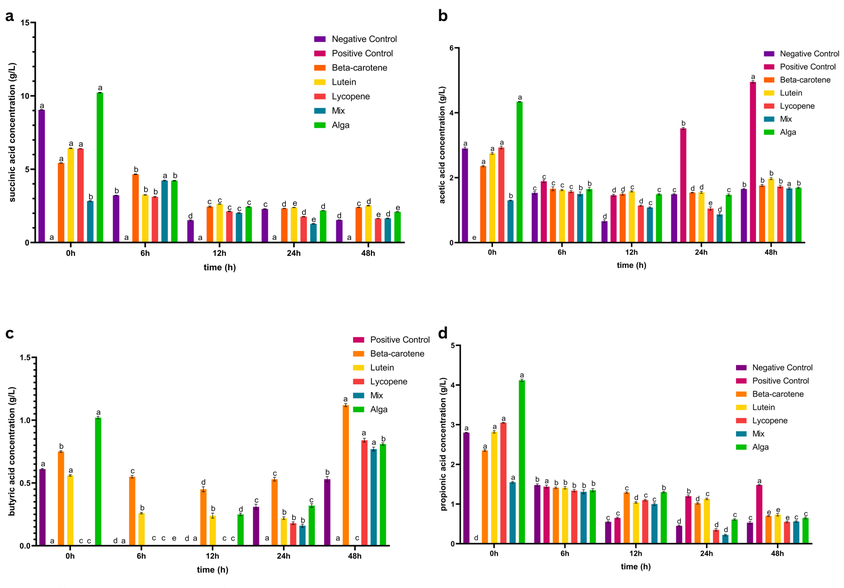


Figure 3. Concentrations (Mean ± SD) of the main organic acids (a–d) released, in g/L, during the 48 h of incubation in the presence of the carotenoid sample groups after simulated GID. Mix: mixed solution of β-carotene, lutein, and lycopene. Alga: *O. pinnatifida*. Different letters mark statistically significant ($p < 0.05$) differences.

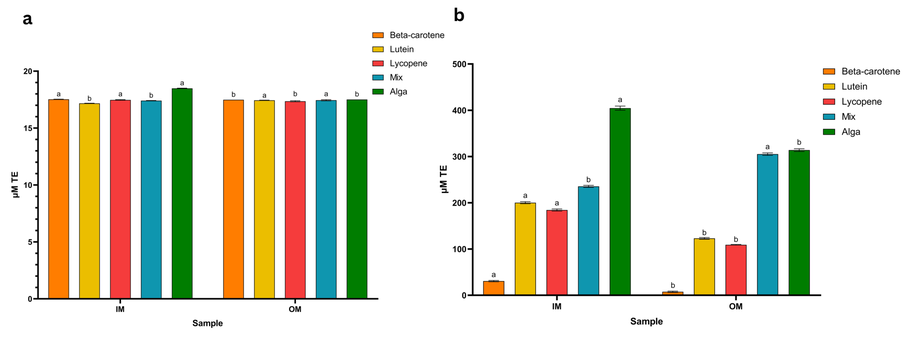


Figure 4. Antioxidant activity (Mean ± SD) by (a) DPPH and (b) ABTS methods of the carotenoids' digested sample groups inside (IM) and outside the membrane (OM). Different letters mark statistically significant ($p < 0.05$) differences within groups.

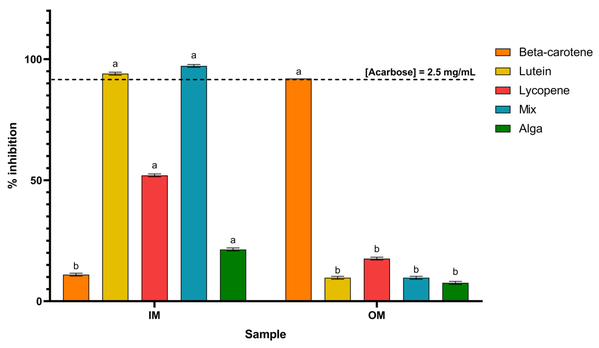


Figure 5. Percentage of α-glucosidase activity inhibition (Mean ± SD) tested with carotenoids' digested samples. Acarbose (2.5 mg/mL) was used as a positive control. IM – inside the membrane; OM – outside the membrane. Different letters mark statistically significant ($p < 0.05$) differences within groups.

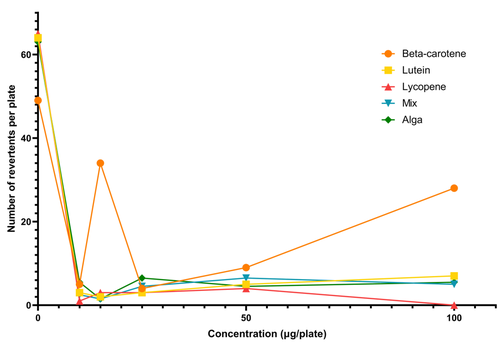


Figure 6. Anti-mutagenicity of carotenoids' sample groups. Results are the means of two determinations ± SD.

CONCLUSIONS:

- Through the *in vitro* digestion simulation, it was observed distinct transformations in carotenoids, indicating intricate changes during digestion;
- Recovery indexes underscored the difficulty in retrieving carotenoids during digestion, highlighting the complexity of their fate in the digestive process.
- Carotenoid's tested groups stimulated the production of organic acids, notably succinic (~6.4 g/L), acetic (~2.75 g/L), butyric (~0.47 g/L), and propionic (~2.78 g/L) acids;
- The analysis of the IM revealed *Bacteroidota*, *Bacillota*, *Pseudomonadota*, and *Actinomycetota* as the main phyla present.
- Carotenoids significantly increased the relative abundance (RA) of the *Lachnospiraceae* family by 77.8% while decreasing the RA of several bacteria, including *Lactobacillus* by 1.27%, *Enterococcus* by 16.3%, *Streptococcus* by 8.80%, and *Bifidobacterium* by 18.3%, which is consistent with previous studies.
- The Mix group demonstrated higher antioxidant activity, particularly when located outside the membrane, compared to other carotenoid groups;
- Lutein and the Mix groups showed effectiveness in anti-diabetic activity, especially when present within the membrane.
- Carotenoid-digested samples exhibited antimutagenic effects, suggesting their potential to support cell development and act as a shield against mutations.

References:

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