Exploring Carotenoid-Intestinal Microbiota Interplay: *In Vitro* Insights into Gastrointestinal Interactions and Health-Enriching Effects

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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 (1). These foods are abundant in **carotenoids**, lipid-soluble phytochemicals (2) known for their health-enhancing properties, including antioxidant, anti-diabetic, and anti-mutagenic effects (3). However, the **intestinal microbiota** (IM) significantly influences the **efficiency of carotenoids** (4).

The IM plays a vital role in the **absorption and metabolism** of carotenoids (4). A balanced diet can modulate the composition of the IM, promoting the growth of beneficial microbes and inhibiting harmful ones (5). The IM also synthesizes and releases various **metabolites**, which can be absorbed into the circulatory system, influencing the host's health (6). These interactions are crucial for understanding **carotenoids' preventive and therapeutic potential**.

Objective: This study aimed to explore the **interaction between carotenoids and the IM** during simulated gastrointestinal digestion and absorption. Three carotenoids (beta-carotene, lutein, lycopene), a pigment mixture (MIX), and the alga *Osmundea pinnatifida* were analyzed. The focus was on understanding how carotenoids affect **bioaccessibility**, **absorption**, **microbial dynamics**, and **organic acid production**. Additionally, the study assessed the **antioxidant, antidiabetic, and antimutagenic properties** of carotenoids, providing insights into their potential health benefits.

1 *In vitro* simulated gastrointestinal digestion (INFOGEST) and absorption

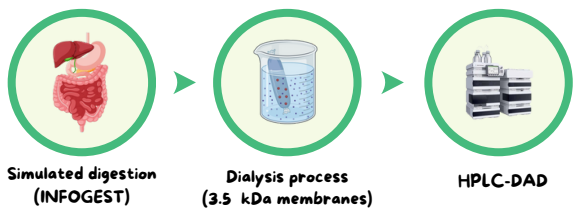


Table 1. Carotenoid profile for the tested conditions at each simulated GIT phase, with their respective concentrations (Mean ± standard deviation (SD)) in mg/L. SSP - simulated salivary phase; SGP - simulated gastric phase; SIP - simulated intestinal phase; IM - dialysis phase inside the membrane; OM - 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	----
	BETA-CRYPTOXANTHIN	9x10-4 ± 6x10-5	LUTEIN	3X10-2 ± 3X10-7
SGP	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	----

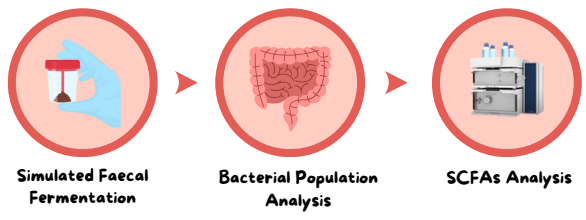
Table 2. Recovery indexes (%) for the carotenoids plain β-carotene and plain lutein at the GIT sampling phases.

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

Main 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.

2 Simulated faecal fermentation, SCFAs and bacterial population analysis



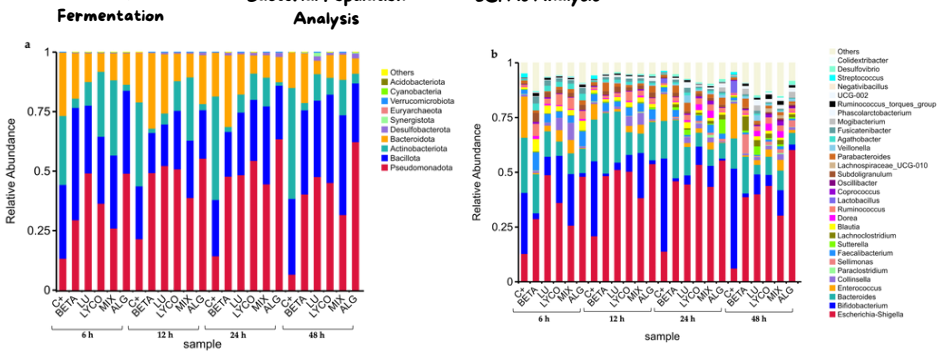


Figure 1. Phyla (a) and genera (b) relative taxonomic abundances from each tested condition at each time-point. (C+: control; BETA: β-carotene; LU: lutein; LYCO: lycopene; MIX: mixed solution of β-carotene, lutein, and lycopene; ALG: *Osmundea pinnatifida*).

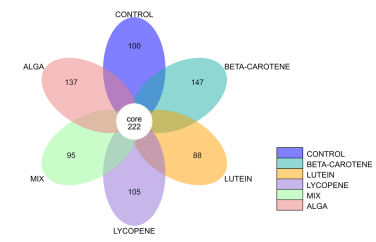


Figure 2. Flower diagram of each sample group. MIX: mixed solution of β-carotene, lutein, and lycopene. Alga: *O. pinnatifida*.

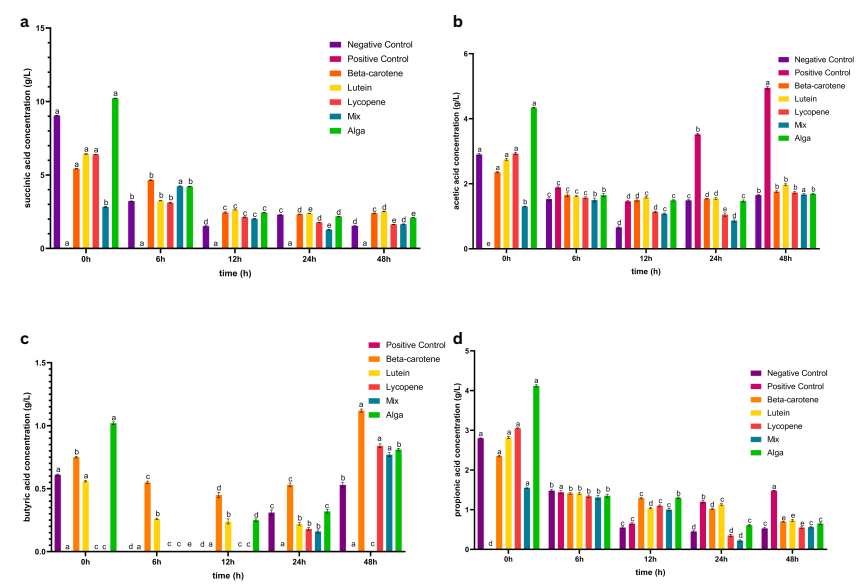
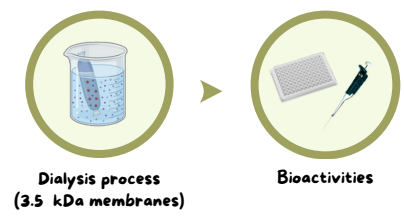


Figure 3. Concentrations (Mean ± SD) of the main organic acids (a–d) released, in g/L, during the 48 h of incubation in the presence of the carotenoid sample groups after simulated GID. Mix: mixed solution of β-carotene, lutein, and lycopene. Alga: *O. pinnatifida*. Different letters mark statistically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

Main conclusions:

- 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.

3 Antioxidant, anti-diabetic and anti-mutagenicity assessment



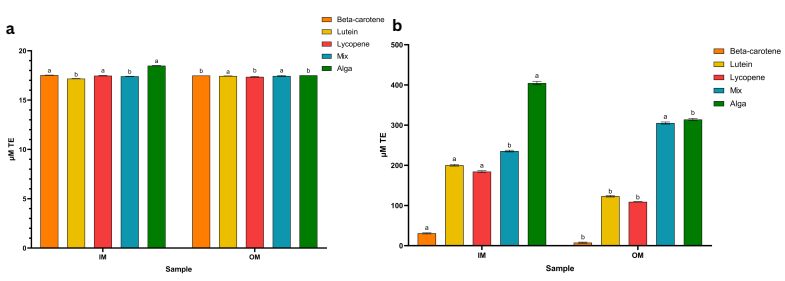


Figure 4. Antioxidant activity (Mean ± SD) by (a) DPPH and (b) ABTS methods of the carotenoids' digested sample groups inside (IM) and outside the membrane (OM). Different letters mark statistically significant ($p < 0.05$) differences within groups.

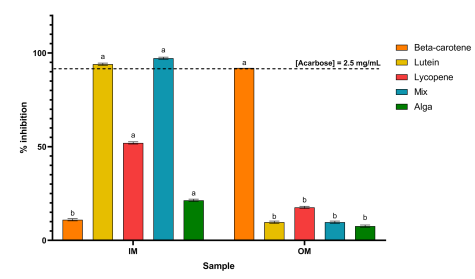


Figure 5. Percentage of α-glucosidase activity inhibition (Mean ± SD) tested with carotenoids' digested samples. Acarbose (2.5 mg/mL) was used as a positive control. IM – inside the membrane; OM – outside the membrane. Different letters mark statistically significant ($p < 0.05$) differences within groups.

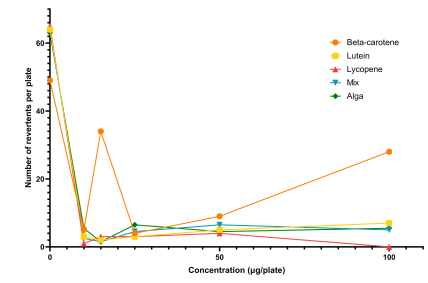


Figure 6. Anti-mutagenicity of carotenoids' sample groups. Results are the means of two determinations ± SD.

Main conclusions:

- 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.

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