pharmaceutics

Supplementary Materials: Polymeric Nanocapsules Containing Fennel Essential Oil: Their Preparation, Physicochemical Characterization, Stability over Time and in Simulated Gastrointestinal Conditions

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Sample Treating before HPLC Injection for Digest Analysis

After simulated gastric digestion: 1) To determine the total *trans*-anethole amount, 500 µL of acetonitrile was added to 500 µL of (neutral) gastric digest. The mixture, after vortexing, was centrifuged (10 min at 10000 × *g*), and, then, 200 µL of the supernatant was diluted with 800 µL of acetonitrile before HPLC injection. 2) To determine the free *trans*-anethole, the sample was centrifuged at 21,00 × *g* as previously depicted in paragraph 2.4.2., and then 500 µL of supernatant was treated as above described for the total amount. The encapsulated *trans*-anethole amount was obtained by difference (total – free).

After simulated digestion (gastric + intestinal): 500 µL of EtOH was added to 500 µL of the methanol-treated supernatant. The mixture, after vortexing, was centrifuged (10 min at 10,000× *g*), then, 400 µL of the supernatant was diluted with 600 µL of acetonitrile, and, after centrifugation (5 min at 3500 × *g*) an aliquot of this mixture was injected in HPLC.

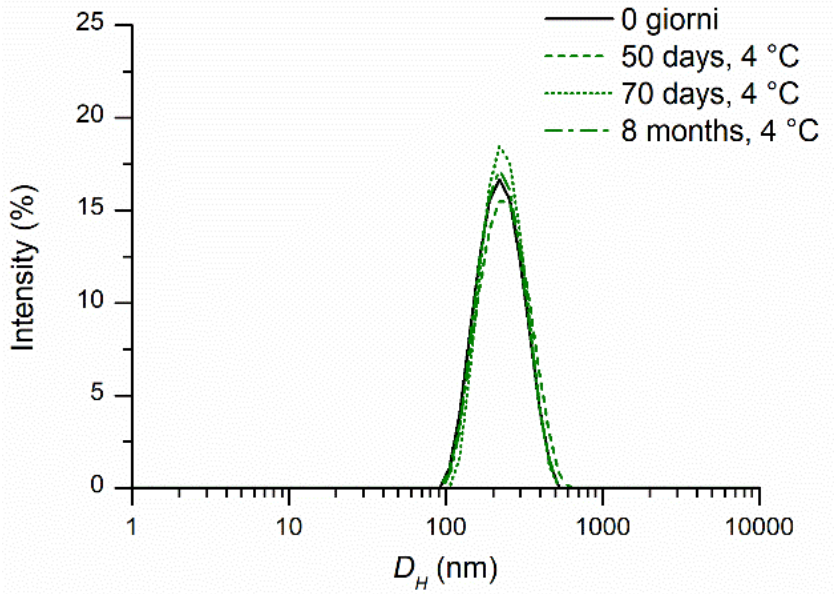

Figure S1. Intensity weighted particle D_H (nm) distribution of FEO-NCs: freshly prepared suspension, stored for 50 days, 70 days, and 8 months at 4 °C.

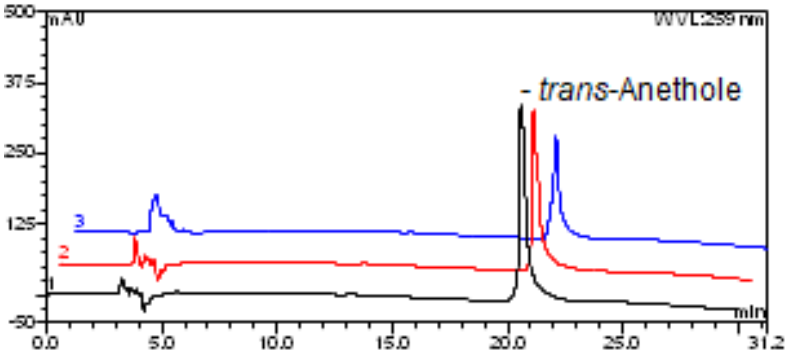

Figure S2. Offset HPLC chromatograms showing the peak (time retention 20.6 min) of the FEO main component (*trans*-anethole) in in vitro simulated digestion experiment: black line sample before the digestion; red line sample after gastric digestion; blue line sample after intestinal digestion.

Table S1. Stability over time of fennel essential oil-loaded nanocapsules (FEO-NCs) at 4 °C.

FEO-NCs	Storage Time (days)				
	0	**7**	**15**	**21**	**30**
Z-average (nm)	210 ± 3 ^{a}	225 ± 4 ^{b}	222 ± 4 ^{b}	220 ± 3 ^{b}	223 ± 6 ^{b}
PDI	0.10 ± 0.04 ^{a}	0.09 ± 0.03 ^{a,b}	0.04 ± 0.02 ^{b}	0.08 ± 0.03 ^{a,b}	0.07 ± 0.03 ^{a,b}
ζ (mV)	−15 ± 2 ^{a}	−15 ± 1 ^{a}	−13 ± 1 ^{a}	−14 ± 2 ^{a}	−15 ± 4 ^{a}
FEO loaded amount (mg/mL)	5.0 ± 0.1 ^{a}	4.8 ± 0.1 ^{a,b}	4.6 ± 0.1 ^{b,c}	4.6 ± 0.1 ^{b,c}	4.5 ± 0.1 ^{c}

Values in the same line with the same superscripts are not significantly different ($p > 0.05$).

Table S2. Stability over time of fennel essential oil-loaded nanocapsules (FEO-NCs) at 40 °C.

FEO-NCs	Storage Time (days)				
	0	**7**	**15**	**21**	**30**
Z-average (nm)	210 ± 3 ^{a}	212 ± 5 ^{a}	210 ± 6 ^{a}	211 ± 2 ^{a}	222 ± 2 ^{b}
PDI	0.10 ± 0.04 ^{a}	0.07 ± 0.03 ^{a}	0.09 ± 0.02 ^{a}	0.08 ± 0.02 ^{a}	0.06 ± 0.04 ^{a}
ζ (mV)	−15 ± 2 ^{a}	−12 ± 1 ^{a}	−16 ± 3 ^{a}	−14 ± 2 ^{a}	−14 ± 4 ^{a}
FEO loaded amount (mg/mL)	5.0 ± 0.1 ^{a}	4.7 ± 0.1 ^{b}	4.6 ± 0.1 ^{b}	4.5 ± 0.1 ^{b}	4.2 ± 0.2 ^{c}

Values in the same line with the same superscripts are not significantly different ($p > 0.05$).

Table S3. Fennel essential oil composition over time of FEO-loaded nanocapsules at 4 °C *.

FEO Component	Storage Time (days)				
(%)	**0**	**7**	**15**	**21**	**30**
limonene	0.37 ± 0.03 ^{a}	0.42 ± 0.07 ^{a}	0.36 ± 0.03 ^{a,b}	0.29 ± 0.04 ^{b}	0.34 ± 0.05 ^{a,b}
fenchone	0.63 ± 0.05 ^{a,b}	0.62 ± 0.04 ^{a}	0.84 ± 0.06 ^{c}	0.72 ± 0.05 ^{b}	0.90 ± 0.07 ^{c}
methyl chavicol	0.40 ± 0.01 ^{a}	0.40 ± 0.01 ^{a}	0.45 ± 0.02 ^{b}	0.44 ± 0.02 ^{b}	0.44 ± 0.02 ^{b}
p-anisaldehyde	0.41 ± 0.02 ^{a}	0.37 ± 0.01 ^{a}	0.69 ± 0.18 ^{b}	0.64 ± 0.13 ^{b}	0.90 ± 0.10 ^{c}
cis-anethole	0.22 ± 0.07 ^{a,b}	0.70 ± 0.13 ^{c}	0.35 ± 0.06 ^{a}	0.35 ± 0.13 ^{a}	0.10 ± 0.06 ^{b}
trans-anethole	94.93 ± 0.23 ^{a,b}	94.20 ± 0.26 ^{a}	96.24 ± 0.58 ^{c}	95.90 ± 1.19 ^{b,c}	96.04 ± 0.36 ^{c}

* Components in percentages lower than 0.2 were not considered. Values in the same line with the same superscripts are not significantly different ($p > 0.05$).

Table S4. Fennel essential oil composition over time of FEO-loaded nanocapsules at 40 °C *.

FEO Component	Storage Time (days)				
(%)	0	7	15	21	30
limonene	0.42 ± 0.06 [a]	0.45 ± 0.06 [a]	0.28 ± 0.09 [b]	0.34 ± 0.07 [a,b]	0.38 ± 0.06 [a,b]
fenchone	0.65 ± 0.04 [a]	0.67 ± 0.06 [a]	0.98 ± 0.03 [b]	0.72 ± 0.07 [a]	0.96 ± 0.06 [b]
methyl chavicol	0.40 ± 0.01 [a]	0.42 ± 0.01 [a]	0.45 ± 0.01 [b]	0.45 ± 0.01 [b,c]	0.47 ± 0.01 [c]
p-anisaldehyde	0.39 ± 0.02 [a]	0.48 ± 0.02 [a]	1.15 ± 0.11 [b]	0.87 ± 0.07 [c]	1.29 ± 0.13 [b]
cis-anethole	0.34 ± 0.11 [a]	0.56 ± 0.09 [a]	0.44 ± 0.17 [b]	0.51 ± 0.18 [a]	0.07 ± 0.03 [b]
trans-anethole	94.66 ± 0.32 [a]	93.82 ± 0.53 [b]	95.02 ± 0.72 [a]	95.80 ± 0.15 [c]	95.35 ± 0.21 [a,c]

* Components in percentages lower than 0.2 were not considered. Values in the same line with the same superscripts are not significantly different ($p > 0.05$).