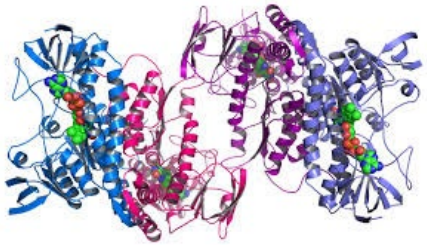
Preliminary study on some innovative rumen-bypass micro-encapsulation techniques for essential oils

Maggiolino A.[1], Denora N.[2], Lopedota A.[2], Di Grigoli A.[3], Ponte M.[3], Ciliberti M.G.[4], Marino R.[1], De Palo P.[1]

[1]Department of Veterinary Medicine, University of Bari, Italy
[2]Department of Farmacy, University of Bari, Italy
[3] Department of Agricultural, Food and Forestry Sciences University of Palermo, Italy
[4] Department of Agriculture, Food, Natural Resources, and Engineering, University of Foggia

How to bypass the rumen

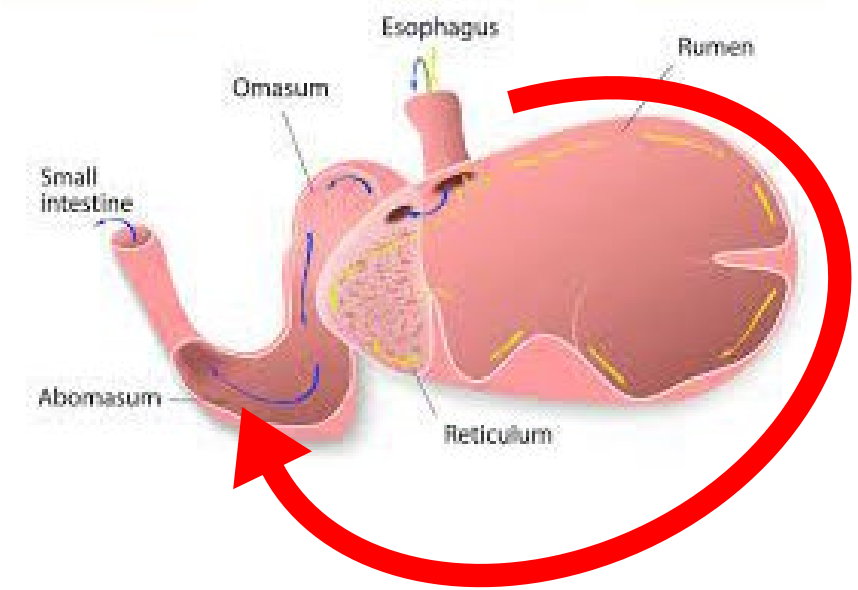
Chemical protection



Proteins or amino acids are complexed with polymers

protective polymers

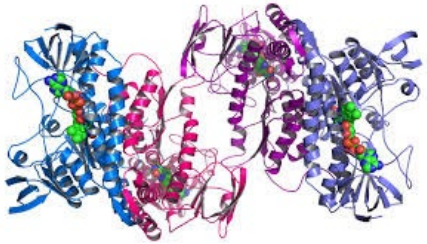
ethyl cellulose



Heat treatments

Some nutrients are spray-dried to create a physical barrier

protective fats



Proteins or amino acids are mixed with fat

Essential oils?

Rumen modifiers

Promote rumen digestive ability

Reduce rumen digestive ability

Modify rumen microbial fermentation

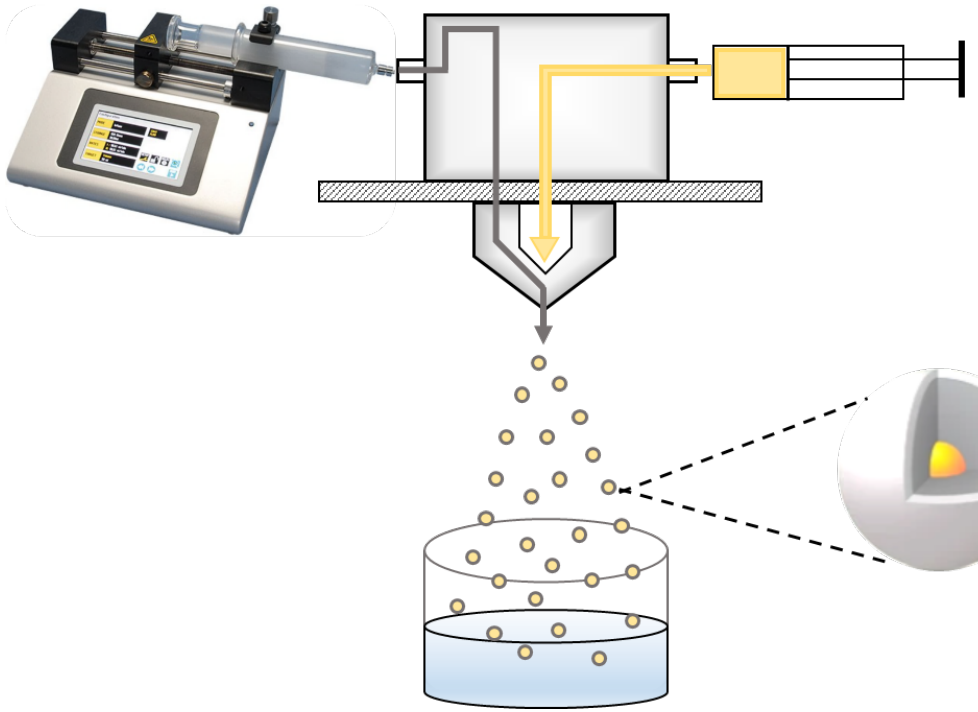
Modify GHG production

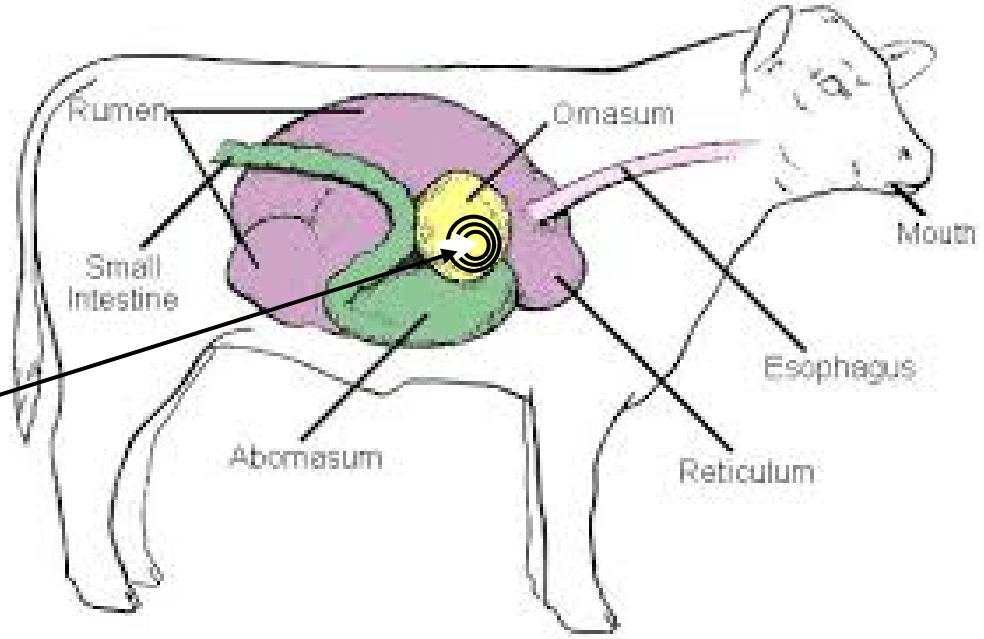
Modify rumen microbial population

Dose-dependent effect often not necessary in the rumen

Aim

Development of a multiparticulate system using prilling/vibration technique for the delivery of essential oil





Shell or core made of a polymer or polymer blend resistant to ruminal degradation, using time-responsive or pH-responsive polymers

Materials and methods

tracer terpene

99.998 % limonene

1. Preparation of the emulsion and its stability study

2. Selection of suitable polymers (for both the core and the shell)

3 Selection of operational parameters (flow rate, frequency, electrodes, nozzle diameters, consolidation bath) and optimization of the production process

4. Characterization of the produced microcapsules

1. Preparation of the emulsion and its stability study

Sodium alginate emulsion (low viscosity) 2% + limonene [1:1 v/v]

Not stable

Sodium alginate emulsion (low viscosity) 2% + limonene [2:1 v/v]

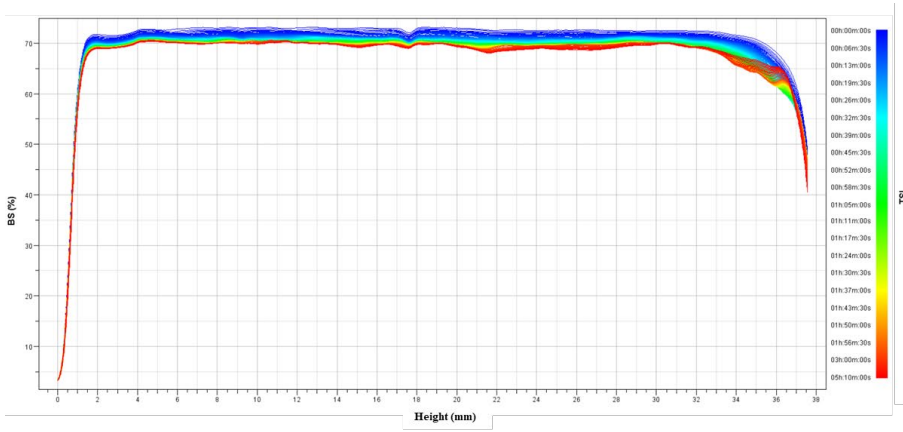
Not stable

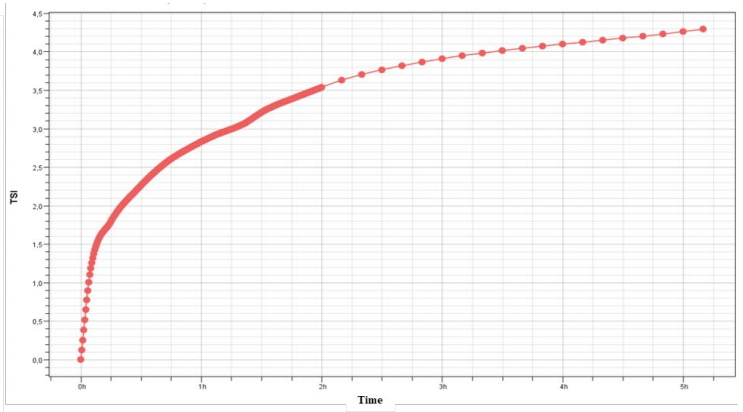
Sodium alginate emulsion (high viscosity) 1% + limonene [1:1 v/v]

More stable

Sodium alginate emulsion (high viscosity) 1% + limonene [2:1 v/v]

Stable



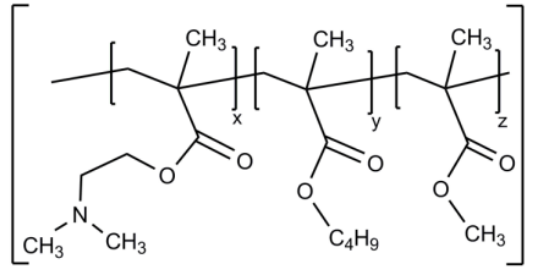


2. **Selection of suitable polymers (for both the core and the shell)**

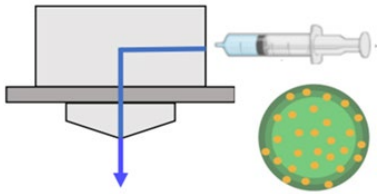
3 **Selection of operational parameters (flow rate, frequency, electrodes, nozzle diameters, consolidation bath) and optimization of the production process**

4. **Characterization of the produced microcapsules**

Eudragit® E PO: Amino Methacrylate Copolymer



Matrix



Sodium alginate emulsion (high viscosity) 1% + limonene [2:1 v/v] + Eudragit EPO [1:2 v/v]

1g beads= 657 µL limonene

Yield = 44.81%

Operational parameters
Nozzle: 450 µm 12mL/min
Frequency: 1200 Hz
Electrode: 1000 V
Consolidation bath: Liquid Nitrogen

REJECTED

Sodium alginate emulsion (high viscosity) 1% + limonene [2:1 v/v] + Eudragit EPO [1:4 v/v]

1g beads= 554 µL limonene

Yield = 70.52%

Operational parameters
Nozzle: 450 µm 7mL/min
Frequency: 1400 Hz
Electrode: 1200 V
Consolidation bath: Liquid Nitrogen

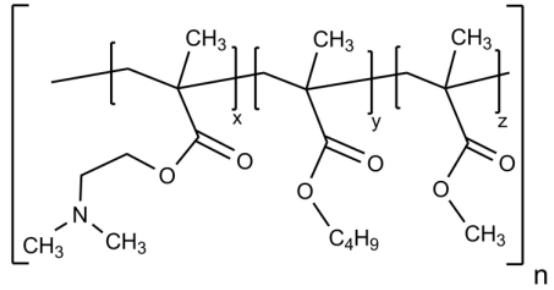
APPROVED

2. **Selection of suitable polymers (for both the core and the shell)**

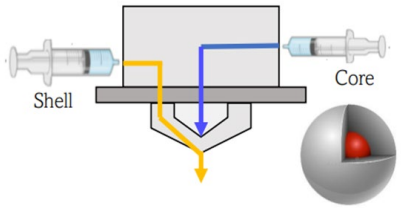
3 **Selection of operational parameters (flow rate, frequency, electrodes, nozzle diameters, consolidation bath) and optimization of the production process**

4. **Characterization of the produced microcapsules**

Eudragit® E PO: Amino Methacrylate Copolymer



Core-shell



Sodium alginate emulsion (high viscosity) 1% + limonene [2:1 v/v]

Shell: Eudragit EPO (10.72% w/v)

1g beads= 558 µL limonene

Yield = 49.97%

Operational parameters

Nozzle core: 450 µm 7mL/min

Nozzle shell: 600 µm 12 mL/min

Frequency: 1200 Hz

Electrode: 1000 V

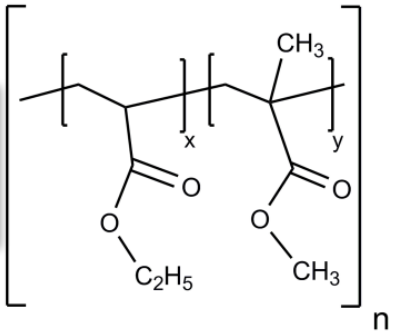
Consolidation bath: Liquid Nitrogen

2. **Selection of suitable polymers (for both the core and the shell)**

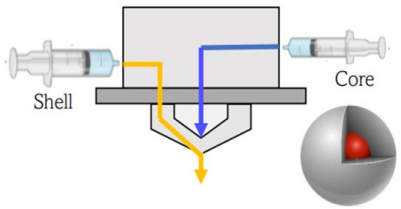
3 **Selection of operational parameters (flow rate, frequency, electrodes, nozzle diameters, consolidation bath) and optimization of the production process**

4. **Characterization of the produced microcapsules**

Eudraguard control®: neutral copolymer based on ethyl acrylate and methyl methacrylate



Core-shell



Core: Sodium alginate emulsion (high viscosity) 1% + limonene [2:1 v/v]

Shell: Eudraguard control (30% w/v)

1g beads= 327 µL limonene

Yield = 74.77% ± 2.74

Operational parameters

Nozzle core: 450 µm 10.5 mL/min

Nozzle shell: 600 µm 4mL/min

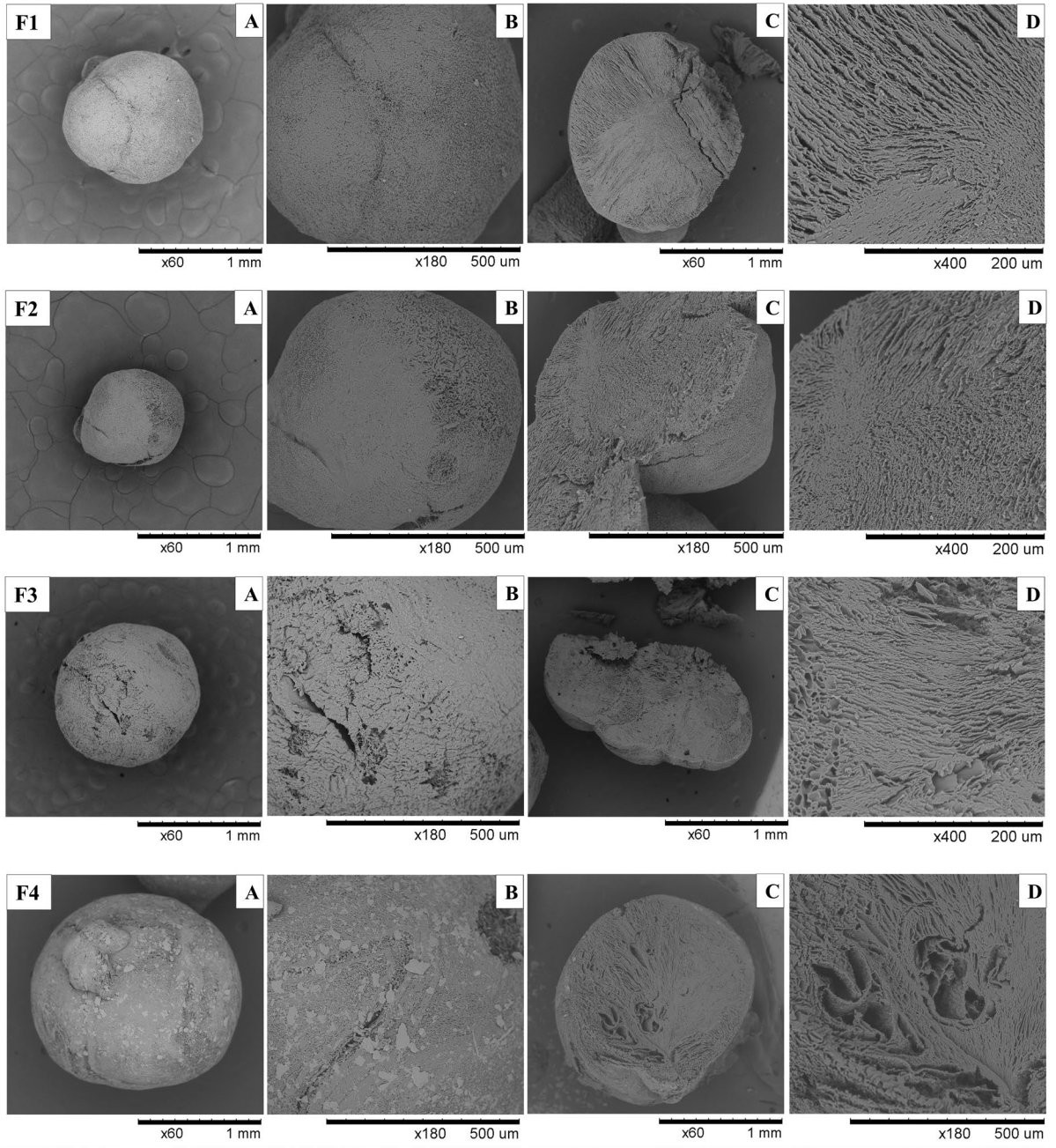
Frequency: 1100 Hz

Electrode: 800 V

Consolidation bath: Liquid Nitrogen

Matrix

Core-shell



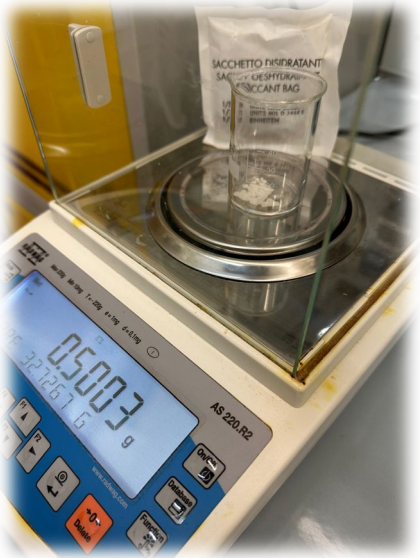
microparticles surface cross-sections

In vitro digestion

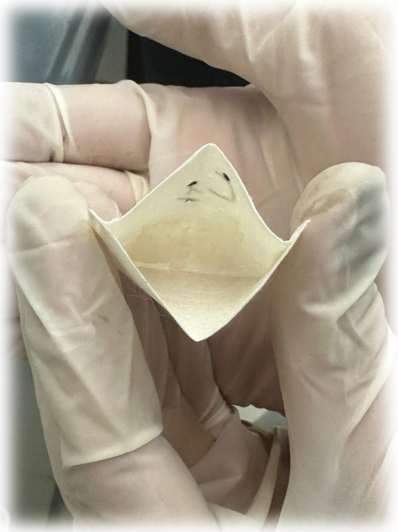
Rumen Fluid

Pool of 4 slaughtered beef

Ankom daisy



0.5 g of microcapsules for each bag



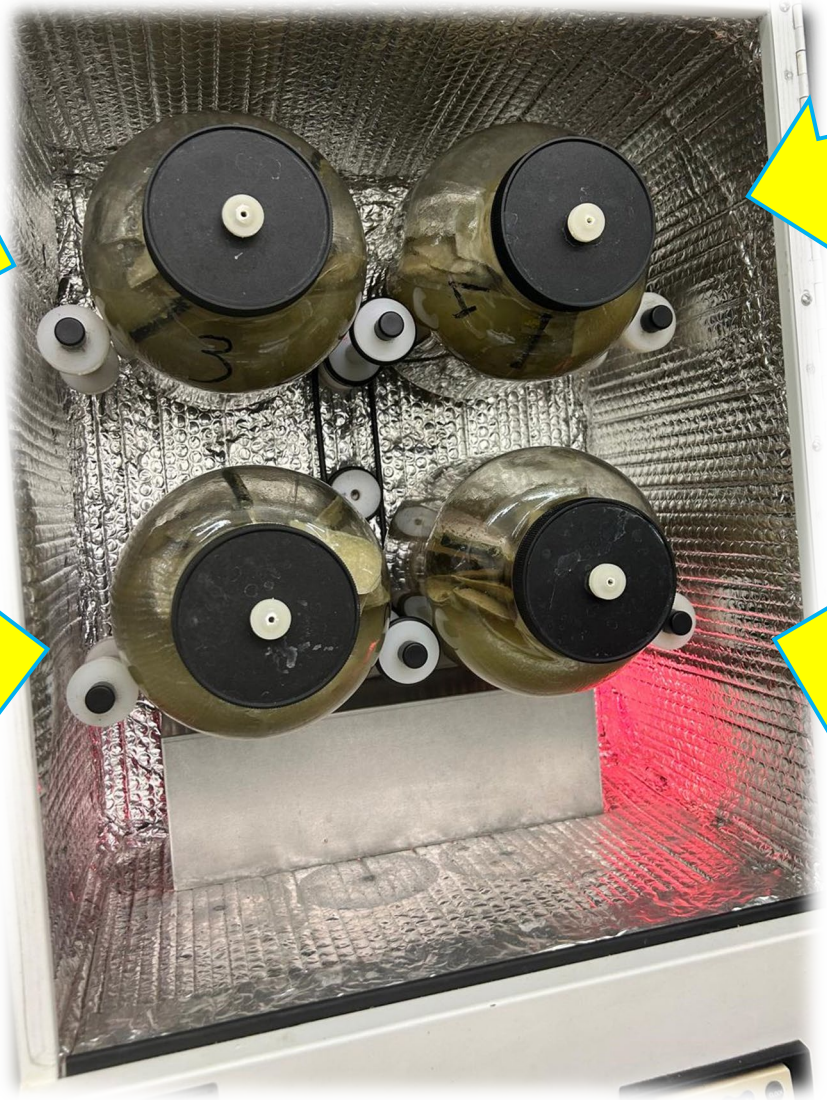
14 bags for each vessel

In vitro digestion

triplicate

6 hours

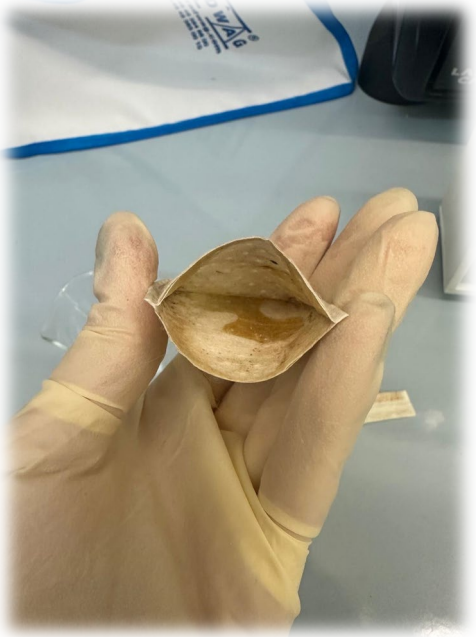
12 hours



18 hours

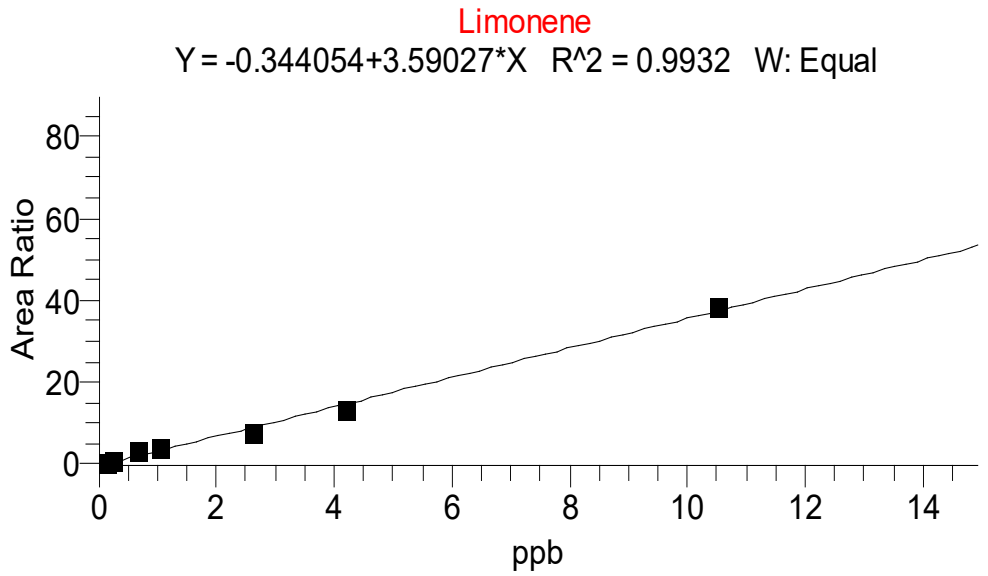
24 hours

Disappearance and limonene quantification



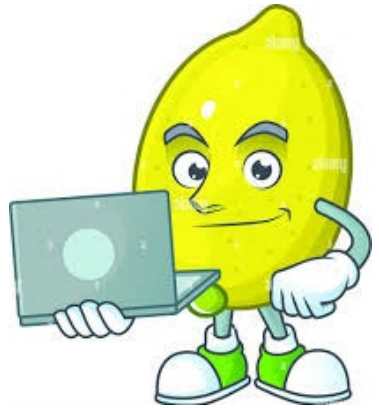
W-W

solid-phase microextraction coupled with GC-MS (SPME/GC-MS)



liquid-liquid extraction with petroleum ether and GC analysis with flame-ionization detection (LLE-GC-FID)

Statistical analysis



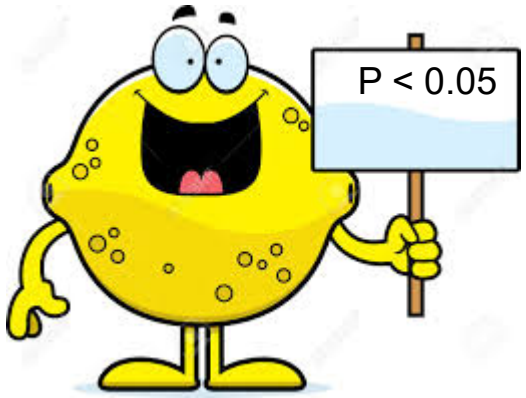
normal distribution

Shapiro-Wilk's test

Analysis of variance (ANOVA)

$$y_{ijk} = \mu + \alpha_i + M_j + \varepsilon_{ijk}$$

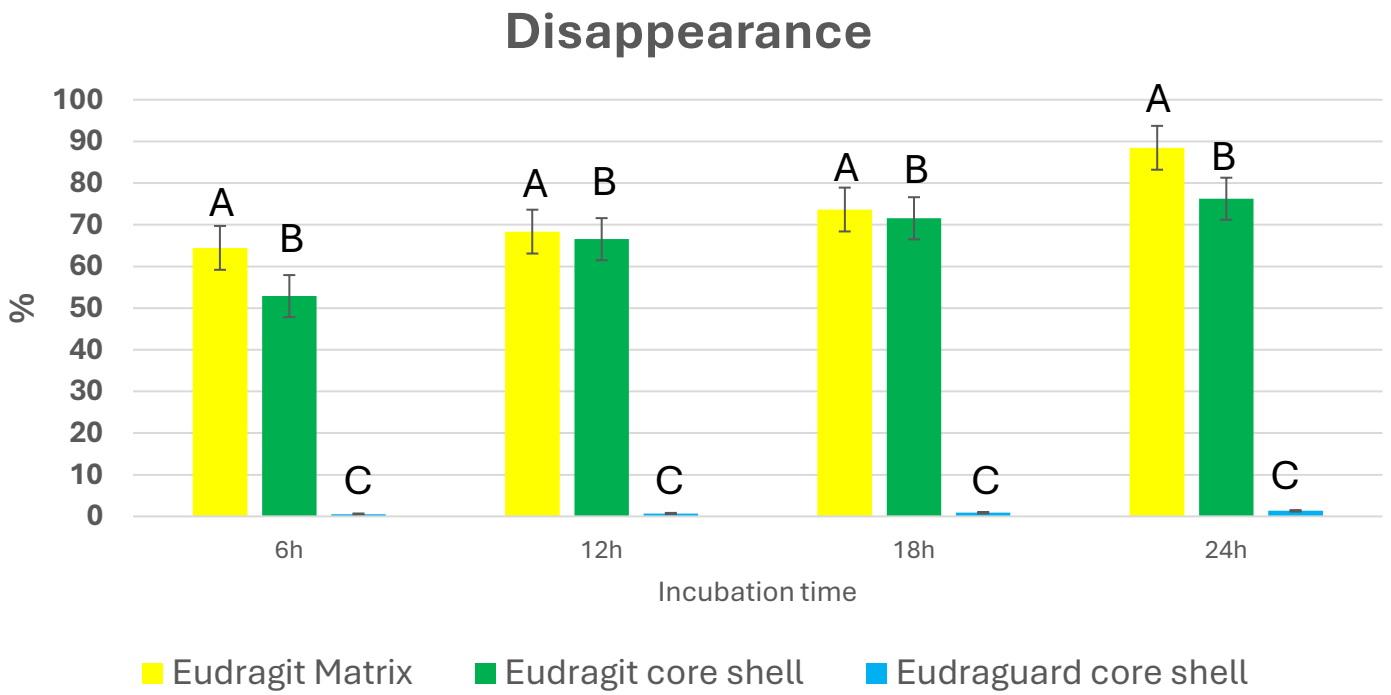
y_{ijk} = parameters (dependent variable)
μ = mean
α_i = replicate random effect ($_i$ = 1,…3)
M = effect of the microcapsule ($_j$ = 1…3)
ε_{ijk} = error



Bonferroni test

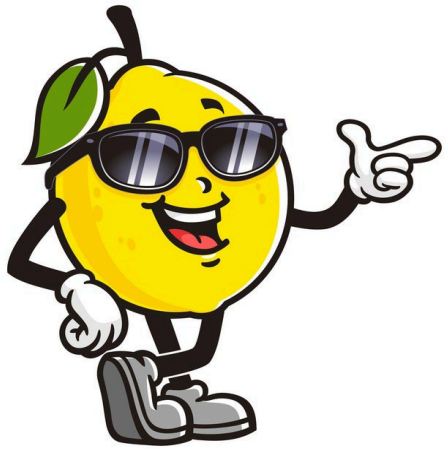
Pairwais comparison between thesis

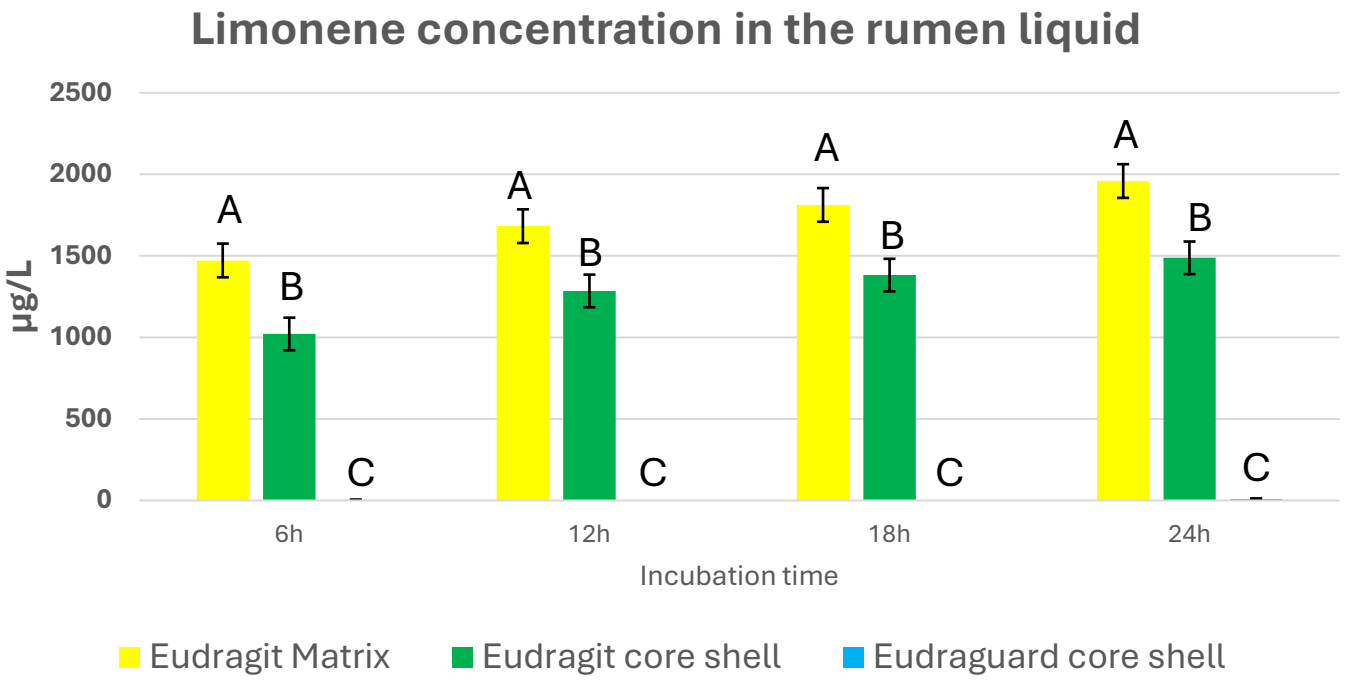
Results



Different letters show statistical differences among thesis: A, B, C, = $P < 0.01$.

Results





Different letters show statistical differences among thesis: A, B, C, = $P < 0.01$.

Concluding

Eudragit® EPO, both in matrix and core shell, shows low resistance in the rumen environment

Eudraguard control® core shell shows great resistance in the rumen environment

Deeper studies should be done to confirm these results and then try with *in vivo* studis

thanks to

UNIVERSITÀ DEGLI STUDI DI PALERMO

Prof. Bonanno Adriana
Prof. Di Grigoli Antonino
Dott. Ponte Marialetizia

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

Prof. Maggiolino Aristide
Prof. De Palo Pasquale
Prof Denora Nunzio
Prof. Lopedota Angela
Dott. Forte Lucrezia
Dott. D'amico Vita
Dott. Calzaretti Giovanna
Dott. Giannico Francesco
Dott. Chiaia Noya Francesca

UNIVERSITÀ DI FOGGIA

Prof. Sevi Agostino
Prof. Albenzio Marzia
Prof. Caroprese Mariuangela
Prorf. Marino Rosaria
Prof. Della Malva Antonella
Dott. Ciliberti maria Giovanna

PNRR-M4C2-linea di investimento 1.1. Progetti di Ricerca di Rilevante Interesse Nazionale (PRIN) – Avviso 1409 del 14/09/2022 – BANDO PRIN 2022 PNRR

Citrus essential oil to cope with heat stress and ensure sustainability in sheep farming Ceo-SUSTAINSHEEP"

THANK
YOU


RELAX
BABY