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Novel dry state co-milling encapsulation of olive leaf extract

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Introduction

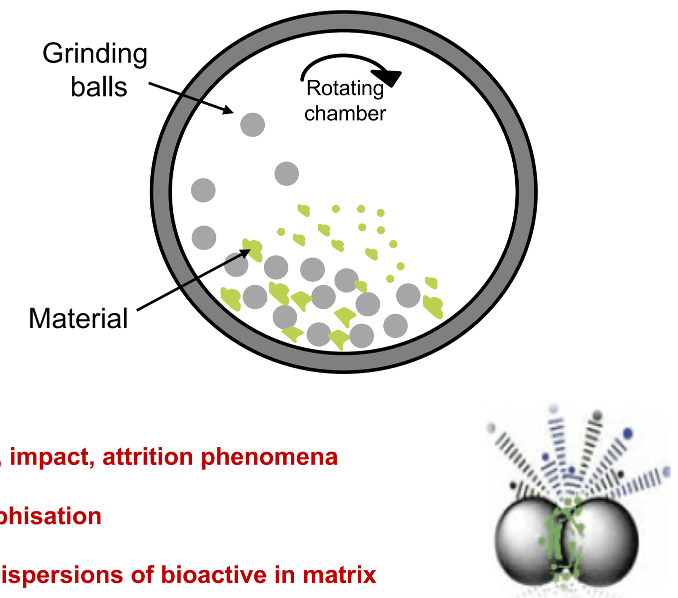
Micro-encapsulation or **nano-encapsulation** is nowadays representing an interesting strategy to enhance the functionality of bioactives and other biomolecules, serving several purposes such as solubility enhancement, increased gastrointestinal absorption or targeted delivery of bioactive compounds (Li et al., 2015). Olive leaves phenolic compounds have been widely studied for their health promoting properties (Martín-Peláez et al., 2013)

Nutritional-pharmaceutical advantages:
- ✓ Increased gastrointestinal absorption
- ✓ Targeted delivery of bioactives

Techno-functional advantages:
- ✓ Solubility enhancement
- ✓ Stability during storage and processing
- ✓ Powder form more applicable and convenient
- ✓ Mask unwanted flavors

• Ball milling

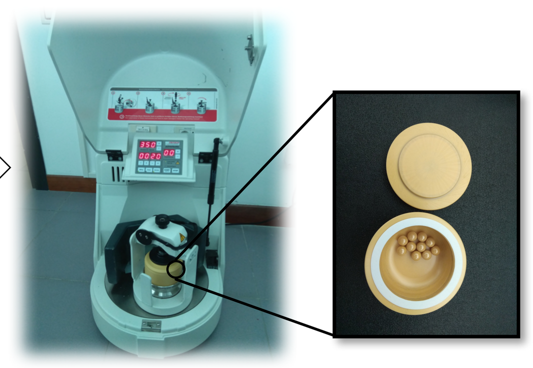
Ball mills are devices that rotate around a horizontal axis where the grinding medium (balls) and material to be ground are subjected to mechanical processes like impact, attrition and shear. A fine molecular dispersion, "molecular alloys", of the active ingredient in the matrix is obtained , along with amorphisation processes similar to those conventionally obtained by spray and freeze-drying (Willart et al., 2006). Modified starch by ball milling has been applied to encapsulate β-carotene (Roa et al.,2016), but no co-milling in the dry state to encapsulate food bioactives has been implemented yet. Thus, co-milling appears to be a promising technique that needs to be further explored for food bioactives encapsulation.



Method

Bioactive — Olive leaf extract(OLE)

Matrix — Maltodextrin(MD) DE 8-10, Trehalose (TR) dihydrate



ZrO_2 grinding bowl (80mL)
ZrO_2 grinding balls (10mm ⌀)

Fixed milling set-up:
- Disk speed: 350 rpm
- Ratio grinding balls:powder (48g:1g)
- 15 min cycles + 5 min pause

Experimental design

Matrix component
- Maltodextrin
- Maltodextrin/Trehalose (90%/10% in weight)

*Ratio matrix:OLE= 1:8.7

Ratio matrix(MD):OLE
- 1:4
- 1:8.7
- 1:15

Milling time
- 0 min (manual mix)
- 30 min
- 60 min
- 120 min
- 180min

Analysis

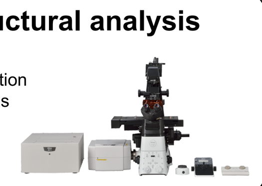
Encapsulation efficiency

$$EE(\%) = \frac{Encap.phenolics\left(\frac{mg}{g}\right)}{Encap.phenolics\left(\frac{mg}{g}\right) + Surface\ phenol}$$

- Total phenolics expressed as mg of gallic acid equivalents (Folin-Cioucalteu assay)

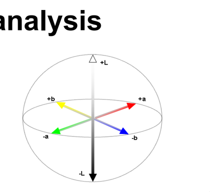
Microstructural analysis

Presence and distribution of phenolic compounds (CLSM microscopy)



Colour analysis

L*a*b* coordinates of encapsulated powders



Results

• Encapsulation efficiency

Table 1. Surface and encapsulated phenolic content, and encapsulation efficiency of encapsulated phenolic fraction in olive leaf co-milled with maltodextrin and maltodextrin+trehalose at 1:8.7 ratio (OLE:MD). Values are means ± SD of triplicate analysis of a single treatment repetition. Different superscripts in a column indicate a significant difference at p<0.05 determined by ANOVA.

Sample	Surphace phenolics (mg GAE g^{-1})	Encapsulated phenolics (mg GAE g^{-1})	Encapsulation efficiency (%)
100% Maltodextrin			
0 min	29.26 ± 1.80 ^{a}	2.38 ± 0.07 ^{a}	7.52 ± 0.30 ^{a}
30 min	13.54 ± 0.26 ^{b}	18.89 ± 0.17 ^{b}	58.24 ± 0.61 ^{b}
60 min	1.51 ± 0.41 ^{c}	29.93 ± 0.26 ^{c}	95.19 ± 1.26 ^{c}
120 min	0.98 ± 0.10 ^{c}	30.87 ± 0.27 ^{c}	96.91 ± 0.30 ^{c}
180 min	0.78 ± 0.13 ^{c}	30.19 ± 0.53 ^{c}	97.47 ± 0.43 ^{c}
90%Maltodextrin 10%Trehalose			
0 min	31.87 ± 1.46 ^{a}	2.87 ± 0.22 ^{a}	8.27 ± 0.36 ^{a}
30 min	8.08 ± 0.14 ^{d}	24.17 ± 0.51 ^{d}	74.92 ± 0.53 ^{d}
60 min	0.84 ± 0.06 ^{c}	30.89 ± 0.31 ^{c}	97.34 ± 0.19 ^{c}
120 min	0.70 ± 0.01 ^{c}	30.65 ± 0.10 ^{c}	97.77 ± 0.03 ^{c}
180 min	0.67 ± 0.01 ^{c}	30.87 ± 0.23 ^{c}	97.87 ± 0.03 ^{c}

Matrix composition and milling time

- ➢ Co-milling effectively resulted in encapsulation of OLE. Encapsulation efficiency was maximized (95-97%) after 60 min milling treatment (**Table 1**)
- ➢ Samples of 90MD:10TR showed a significantly higher encapsulation efficiency at 30 min compared to those with 100MD matrix. More repetitions of each treatment are required to confirm these differences.

Table 2. Surface and encapsulated phenolic content and encapsulation efficiency of encapsulated phenolic fraction in olive leaf co-milled with maltodextrin at different ratio OLE:MD (1:4, 1:8.7 and 1:15). Values are means ± SD of triplicate analysis of a single treatment repetition. Different superscripts in a column indicate a significant difference at p<0.05 determined by ANOVA.

Sample	Surphace phenolics (mg GAE g^{-1})	Encapsulated phenolics (mg GAE g^{-1})	Encapsulation efficiency (%)
Ratio 1:15			
0 min	17.32 ± 0.65	1.65 ± 0.06	8.70 ± 0.29 ^{a}
60 min	0.64 ± 0.15	19.14 ± 0.31	96.76 ± 0.71 ^{b}
180 min	0.65 ± 0.02	19.27 ± 0.30	96.75 ± 0.10 ^{b}
Ratio 1:8,7			
0 min	29.26 ± 1.80	2.38 ± 0.07	7.52 ± 0.30 ^{a}
60 min	1.51 ± 0.41	29.93 ± 0.26	95.19 ± 1.26 ^{b}
180 min	0.78 ± 0.13	30.19 ± 0.53	97.47 ± 0.43 ^{b}
Ratio 1:4			
0 min	52.93 ± 2.95	5.14 ± 0.57	8.84 ± 0.74 ^{a}
60 min	6.66 ± 0.15	49.87 ± 0.56	88.21 ± 0.25 ^{c}
180 min	1.21 ± 0.08	55.02 ± 0.29	97.85 ± 0.14 ^{b}

Ratio matrix (MD):OLE

- ➢ Encapsulation efficiency showed a similar trend, except for ratio 1:4 that showed a slightly lower encapsulation after 60 min, but longer treatments resulted in maximized encapsulation (**Table 2**)

• Microstructural analysis

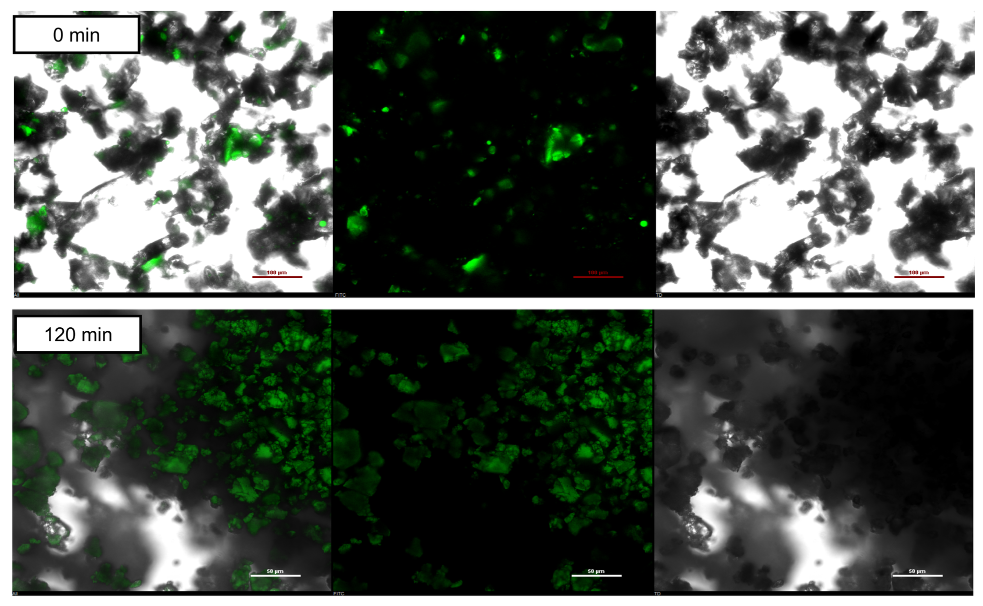


Figure 1. Images of manual mix (0 min milling) and 120 min milled samples of olive leaf extract and maltodextrin with 1:8.7 ratio

- ➢ Co-milled powders result as fine solid-state homogenous dispersion of olive leaf extract in the maltodextrin matrix while non-milled sample (0 min) shows unhomogeneously distributed phenolic extract (**Figure1**)
- ➢ Olive leaf extract and manual mix (0 min co-milling) samples showed the presence of green liquid droplets in the micro-images due to the quickly absorbed moisture or organic solvent present in nail polish used during sample preparation. This, on the contrary, did not occur in OLE-comilled samples, indicating a protective effect of the maltodextrin matrix over the dispersed phenolic extract upon environmental humidity and/or solvents.

• Colour

Table 3. Colour coordinates of olive leaf extract and co-milled samples at ratio 1:8.7 (OLE:MD). Values are means ± SD of triplicate analysis. Different superscripts in a column indicate a significant difference at p<0.05 determined by ANOVA.

	Colour		
Sample	L*	a*	b*
Olive leaf extract	37.13 ± 0.48	4.36 ± 0.04	21.99 ± 0.09
100% Maltodextrin			
0 min	47.42 ± 0.69 ^{a}	1.98 ± 0.38 ^{a}	15.57 ± 0.76 ^{a}
30 min	52.53 ± 0.04 ^{b}	0.00 ± 0.02 ^{b}	15.78 ± 0.17 ^{a}
60 min	51.50 ± 0.60 ^{b}	0.14 ± 0.04 ^{b}	16.95 ± 0.23 ^{b}
120 min	51.03 ± 1.02 ^{b}	0.07 ± 0.03 ^{b}	17.12 ± 0.14 ^{b}
180 min	50.92 ± 1.10 ^{b}	0.06 ± 0.03 ^{b}	16.71 ± 0.07 ^{b}
90%Maltodextrin 10%Trehalose			
0 min	48.88 ± 0.42 ^{a}	1.63 ± 0.11 ^{a}	15.79 ± 0.25 ^{a}
30 min	52.55 ± 0.23 ^{b}	0.10 ± 0.03 ^{b}	16.38 ± 0.32 ^{ab}
60 min	51.96 ± 0.27 ^{b}	0.11 ± 0.05 ^{b}	16.76 ± 0.36 ^{ab}
120 min	51.10 ± 0.31 ^{b}	0.15 ± 0.03 ^{b}	17.19 ± 0.33 ^{b}
180 min	50.98± 0.35 ^{b}	0.11 ± 0.04 ^{b}	16.82 ± 0.26 ^{b}

- ➢ Colour analysis of OLE co-milled samples highlights that milling treatment resulted in lighter (L*values) powders compared to non-milled samples, although no significant differences were observed between different milling times.
- ➢ Values of a* were lower for milled samples, and b* values were higher compared to non-milled samples.
- ➢ Overall, milling of OLE with maltodextrin/trehalose resulted in powders with lighter colours, thereby masking the brownish-yellowish colour of olive leaf extract. This can be of interest for further food applications where a change in colour of the final product is not desirable.

Reference

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Conclusions

- ➢ High energy milling applied using a planetary ball mill is a simple and easy to operate process, with high potential to produce co-milled bioactive encapsulates.
- ➢ Encapsulation of olive leaf extract was maximized after 1h treatment, producing a fine dispersion and distribution of OLE in the internal surface of the matrix. Higher encapsulation(60min-180min) also seemed to protect the OLE fraction from ambient moisture/solvents as observed during microscopy analysis.
- ➢ Milling resulted in powders with lighter yellow-brownish, thereby masking the OLE dark brown colour, that can also be of interest for further food applications