Nanoencapsulation of food ingredients: From macromolecular nanostructuring to smart delivery systems

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a simple story
about a simple concept
that simply works
Functional foods:
Smart protection and delivery of bioactives

STABILITY
Stable against heat, pH and oxidation in food processing

TASTE & COLOR
No unpleasant taste or color

SAFETY
Mild on the stomach because of its insolubility in gastric juices

BIOAVAILABILITY
Sustained release, high absorption and bioavailability
Outline

• Proof of concept – CLA
• What is the effect of the guest molecule?
• Can we use the system for non FA’s compounds?
• The continuous process
• Enhanced bioavailability
• Conclusion
The system of interest: Amylose molecular complexes with LMW compounds

Different # of Glucose per turn

In / Between the helixes
The concept:
Intimate guest-host interaction will prove useful, for…

Oxidative & Thermal stability

Stable in acid stomach

Bioavailability

Released in GI and colon

P. Lebail et al., Carbohydrate Polymers, 43 (2000), 317-326.
How do we make them:

- Alkali solution
- Acidification
- DMSO solution
- Dilution into water
- Complexes

Amylose → Form I → Form II
Our model molecule: CLA

**CLA:** a group of polyunsaturated fatty acids that exist as positional and stereoisomers of conjugated dienoic octadecadienoate (18:2).

**Physiological properties:**
- antiadipogenic,
- antidiabetogenic,
- anticarcinogenic,
- antiatherosclerotic,
- effects on bone formation,
- ...

XRD of amylose-CLA complexes produced by two complexation methods at 3 temperatures


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Thermal stability of amylose-CLA complexes: DSC

<table>
<thead>
<tr>
<th></th>
<th>$T_{\text{cyst}}$ ($^\circ$C)</th>
<th>$T_{\text{onset}}$ ($^\circ$C)</th>
<th>$T_m$ ($^\circ$C)</th>
<th>$\Delta H$ (J/gr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KOH / HCl</td>
<td>90</td>
<td>78.9 ± 2.8</td>
<td>89.6 ± 2.2</td>
<td>7 ± 4.1</td>
</tr>
<tr>
<td>KOH / HCl</td>
<td>60</td>
<td>90.8 ± 6.4</td>
<td>93.6 ± 4.1</td>
<td>12.5 ± 3.4</td>
</tr>
<tr>
<td>KOH / HCl</td>
<td>30</td>
<td>85.7 ± 0.4</td>
<td>89.9 ± 0.6</td>
<td>9.7 ± 1.2</td>
</tr>
<tr>
<td>DMSO</td>
<td>90</td>
<td>79 ± 5.1</td>
<td>94.1 ± 3.8</td>
<td>17.4 ± 2.7</td>
</tr>
<tr>
<td>DMSO</td>
<td>60</td>
<td>87.1 ± 5.2</td>
<td>92.3 ± 2.2</td>
<td>12.3 ± 4.1</td>
</tr>
<tr>
<td>DMSO</td>
<td>30</td>
<td>81.2 ± 0.9</td>
<td>88.9 ± 0.1</td>
<td>13 ± 2.1</td>
</tr>
</tbody>
</table>

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What about the size?
With amylose: ~400 nm particles

AFM images (4.5μmX4.5μm) of V-amylose nanocapsules hosting Linoleic Acid produced via DMSO method [A] or via acidification [B].
Outline

• Proof of concept – CLA

• **What is the effect of the guest molecule?**

• Can we use the system for non FA’s compounds?

• The continuous process

• Enhanced bioavailability

• Conclusion
Change in degree of crystallinity

X-ray diffraction of V type amylose inclusion complexes hosting Stearic acid (SA), Oleic acid (OA) and Linoleic acid (LA) made by acidification method (a) or DMSO method (b). Crystalline polymorphism of V-amylose hosting stearic acid denoted as polymorph 1 and polymorph 2.

Particle size distribution curves of suspensions containing V-amylose inclusion complexes hosting Stearic acid (SA), Oleic acid (OA) or Linoleic Acid. Complexes were produced via DMSO method. Curves calculated based on the light scattering of the samples processed using the general Fraunhofer model weighted by volume.

A clear difference in FA’s mobility in the complex

13C CP/MAS solid state NMR spectra of amylose complexes hosting Stearic acid (SA), Linoleic Acid (LA) and Conjugated Linoleic Acid (CLA) made DMSO method at 90oC.

Zabar, Lesmes, Schmidt, Shimoni, Bianco-Peled, Food Hydrocolloids (2009)
The time scale is of interest:

Potential colonic delivery

Enzymatically induced release profile of stearic acid from two types of crystalline polymorphs (termed V1 and V2) of V-type amylose nanocapsules prepared via DMSO method. Controls were achieved by suspending samples in PBS for similar periods of time. Values are expressed as replicate means (*signifies p<0.05; ** p<0.01; ***p<0.001).

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Other nutrients / bioactives? – polyphenols?

- **Isoflavones** - subclass in the flavonoids family.
- Beneficial health effects:
  - Reducing the risk of cardiovascular diseases.
  - Lowering rates of prostate, breast and colon cancers.
  - Improving bone composition.
- Recommended dose: 40-50 mg daily*.

- Genistein

- Genistin

X-ray Diffraction- HACS

HACS genistein complexes

Amylose genistein complexes

HACS no guest

Glucose genistein molar ratio

** Glucose genistein molar ratio = (amylose content in the complex/Mw (glucose))
  (genistein content in the complex /Mw (genistein))

- Reported glucose-to-guest molar ratio: 8 - 25.
Genistein release in simulated stomach

- PBS: pH=6.9, 37 °C, 24h.
- HCl: pH=2, 37 °C, 2h.


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Genistein release in simulated intestinal conditions

![Intestine](image)

- **HACS**
- **Amylose**

**Genistein released (%)**

- **PBS** (control)
- **HCl** (Stomach simulated conditions)
- **Pancreatin** (Intestine simulated conditions)

*Pancreatic solution: pH=6.9, 140 units/ml, 37 ºC, 24h.*

Kinetics of Genistein Release in Simulated Intestinal Conditions

HACS

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Atomic Force Microscopy (AFM) of Genistein-Amylose Complexes

Undiluted sample

Diluted sample (X50)

4.5μm X 4.5μm; Tip: CSC21 No/Al – contact mode

• The aggregation is reversible.
• Mean particle size of 200 ± 90 nm.
Colloidal stability

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The test-tube concept works!

But it is only a test tube...
Pilot scale manufacturing: cheap starch + sub micron capsules

Proof of concept and some more…

- Gelatinization is critical.
- Significant size reduction.
- Controlled release.

Lesmes, Barchechath & Shimoni, *Innovative Food Science and Emerging Technologies*, 2008,

*Laboratory of Functional Foods, Nutraceuticals, and Food Nanosciences*
What’s now?

Development of foods containing nanoencapsulated ingredient

Enhanced bioavailability

A European Commission funded project within the Seventh Framework Programme
Call Identifier: FP7-SME-2007-1
Project No: 222006
From Lab-scale to Pilot-scale processing

Preparation of Solution

Acidification

Drying of complexes
SEM – microparticles containing w-3

magnification 1000 (1) and 10000 (2) magnification, bar 100μm and 10μm respectively.

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Stability under different temperatures

The onset temperature, melting temperatures and enthalpies of DSC endotherms for different samples of HACS with and without omega-3

All values were normalized to sample dry weight and expressed as mean±SD (n≥2)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Event 1</th>
<th>Event 2</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$T_{\text{onset}}$ (°C)</td>
<td>$T_{\text{melting}}$ (°C)</td>
</tr>
<tr>
<td>HACS (processed)</td>
<td>85.5±1.3</td>
<td>100±1.6</td>
</tr>
<tr>
<td>Flax seed oil</td>
<td>86.1±2.5</td>
<td>94.7±0.3</td>
</tr>
<tr>
<td>pH=5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl esters</td>
<td>82.6±1.3</td>
<td>94±0.6</td>
</tr>
<tr>
<td>pH=5.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish oil</td>
<td>80.4±2</td>
<td>90.7±4.5</td>
</tr>
<tr>
<td>pH=3.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Stability under different temperatures

Omega-3 profile of ‘Flax seed oil’ complexes during high temperature treatment 60°C (A) and 90°C (B).
Shelf life testing

Omega-3 profile of ‘Flax seed oil’ complexes during storage at temperature 4°C, 25°C and 37°C
Amount of omega-3 present after simulated stomach conditions (pH=2, 2 hours), PBS (pH=6.9, 3 hours) and enzymatic digestion (α-amylase (50U/ml), pH=6.9, 3 hours) in samples Flax seed oil pH=5.7
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- **Enhanced bioavailability**
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Animals, Diets and Treatment

10 male Sprague-Dawley rats (220-250g)

Rats were placed in metabolic cages and were fasted overnight (free access to water).

After 12 h

- HACS-genistein COMPLEXES
  30 mg genistein /kg of body weight

- HACS-genistein MIXTURE
  30 mg genistein /kg of body weight
HACS-genistein complexes increases genistein bioavailability

- Higher in complexed genistein
- Lower in complexed genistein
- HACS-genistein complexes increase genistein bioavailability

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Conclusion

V-amylose offers the possibility of nanoencapsulating: poly-unsaturated fatty acids, phenolic compounds, aroma chemicals, and drugs.

Complexes form particles with diameter of 400nm-30μm.

Complexation enables pursuing an array of applications.

Using continuous up-scale process demonstrate the feasibility of forming such systems from a cheap and commercially viable food grade product.

Complexation enhanced the bioavailability of genistein in rats.
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