Microencapsulation of Probiotics

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Outline

• Probiotic Benefits and Claims in Products
• Applications in Food/Feed and Beverages
• Keys to Delivery (Heat, Humidity, Oxygen)
• Review of Various Encapsulation Methods
Probiotics vs. Prebiotics

Probiotics
Live microbial supplements that have beneficial effects on the consumer

Prebiotics
Feed the good that already populate the digestive system

“Live microorganisms which, when administered in adequate amounts confer a health benefit on the host”
Human Microbiome Project


NIH HUMAN MICROBIOME PROJECT

Probiotic Benefits: Promoting Digestive Health (Probiotics and Prebiotics)

Digestive health is the most studied and documented benefit of probiotics.

Eckburg, PB et al. Science 2005:308;1635-8
Probiotic Benefits: Supporting the Immune System

Probiotics release soluble factors and antimicrobial compounds triggering signaling cascades that activate underlying immune cells.

A clinical study demonstrated a significant reduction in the frequency of atopic diseases in infants supplemented with *Lactobacillus GG* (*Kalliomäki et al.,* 2001, 2007).

Probiotics relieve and improve conditions of those suffering from autoimmune diseases, such as rheumatoid arthritis, type 1 diabetes, multiple sclerosis, lupus psoriasis etc. *(See a Review Article by Herichr, R. and Levkut M. - Vet. Med. – Czech, 47, 2002 (6): 169–180)*
Probiotic Claims in Dietary Supplements

- Structure/function statements in dietary supplement may not claim to diagnose, mitigate, treat, cure or prevent a disease.

- Dietary supplements making disease claims are deemed to be drugs.

- Structure/function statements for pre-probiotics could be related to immune function, intestinal health, bowel function.
Probiotic Supplement Challenge: Packaging Cost and Storage Conditions

• Preservation (storage shelf life)
  • Humidity
  • Temperature
  • Oxidation
• Gastric Protection
• Post Gastric Release
Probiotic Challenges in Food: A Wide Variety of Products

Process and Storage Challenges

Treats

Ice Cream

Beverages (Cold or Hot)

Yogurts

Baby Cereals
Shelf Storage Challenge: Water Activity

From Crittenden et al., Unpublished data (Food Science Australia)
Can Encapsulation Improve Probiotic Survival in Dry Products?

“…There is no quick fix for maintaining cell survival under the high moisture conditions encountered by bacteria in food matrices.”

…A multifaceted approach is warranted incorporating emerging knowledge of probiotics… as well as preservation technologies designed to improve and maintain probiotic survival during food processing, storage, and consumption.”
Water-vapor and Oxygen Permeability of Polymer Films

None of the bio or synthetic polymers is 100% impermeable.

Polymers having good WVP may not necessarily have good O₂P

### Table 4. Comparison of the oxygen permeability of edible polymer films and conventional synthetic polymer films

<table>
<thead>
<tr>
<th>Film Type</th>
<th>Test conditions</th>
<th>Permeability (nm³·cm⁻²·s⁻¹·Pa⁻¹)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose-based:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>24°C, 50% RH</td>
<td>97</td>
<td>21</td>
</tr>
<tr>
<td>HPMC</td>
<td>24°C, 50% RH</td>
<td>272</td>
<td>21</td>
</tr>
<tr>
<td>MC</td>
<td>25°C, 52% RH</td>
<td>90</td>
<td>22</td>
</tr>
<tr>
<td>Starch-based:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylo-oligosaccharide starch</td>
<td>25°C, 50% RH</td>
<td>&lt;65</td>
<td>23</td>
</tr>
<tr>
<td>Hydroxypropylated amylo-oligosaccharide starch</td>
<td>25°C, 50% RH</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Protein-based:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>RT, 6% RH</td>
<td>&lt;0.004–0.05</td>
<td>25</td>
</tr>
<tr>
<td>Collagen</td>
<td>RT, 6% RH</td>
<td>21.3</td>
<td>25</td>
</tr>
<tr>
<td>Collagen</td>
<td>RT, 6% RH</td>
<td>21.3</td>
<td>25</td>
</tr>
<tr>
<td>Zein:PEG+glycerol (2.6:1)</td>
<td>25°C, 50% RH</td>
<td>38.7–90.3</td>
<td>26</td>
</tr>
<tr>
<td>Glutens:glycerol (2.5:1)</td>
<td>25°C, 50% RH</td>
<td>6.1</td>
<td>27</td>
</tr>
<tr>
<td>Soy protein isolate:glycerol (2.4:1)</td>
<td>25°C, 50% RH</td>
<td>6.1</td>
<td>28</td>
</tr>
<tr>
<td>Whey protein isolate:glycerol (2.3:1)</td>
<td>25°C, 50% RH</td>
<td>76.1</td>
<td>29</td>
</tr>
<tr>
<td>Whey protein isolate:sorbitol (2.3:1)</td>
<td>25°C, 50% RH</td>
<td>3.3</td>
<td>29</td>
</tr>
<tr>
<td>Synthetics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDPE</td>
<td>23°C, 50% RH</td>
<td>18.70</td>
<td>6</td>
</tr>
<tr>
<td>HDPE</td>
<td>23°C, 50% RH</td>
<td>32.97</td>
<td>6</td>
</tr>
<tr>
<td>Polyester</td>
<td>23°C, 50% RH</td>
<td>13.6</td>
<td>30</td>
</tr>
<tr>
<td>EVOH (70% VOH)</td>
<td>23°C, 50% RH</td>
<td>0.12</td>
<td>6</td>
</tr>
<tr>
<td>EVOH (70% VOH)</td>
<td>23°C, 50% RH</td>
<td>0.12</td>
<td>6</td>
</tr>
<tr>
<td>PVDC-based films</td>
<td>23°C, 50% RH</td>
<td>0.4–5.1</td>
<td>6</td>
</tr>
</tbody>
</table>

*See Table 2 for polymer abbreviations.

### Table 18.2 Properties of selected synthetic and biopolymer films for comparison to myofibrillar protein films (Krocha et al., 1994; Cuq, 2002)

<table>
<thead>
<tr>
<th>Film</th>
<th>Tensile strength (MPa)</th>
<th>Elongation (%)</th>
<th>WVP* (×10⁻¹² mol·m⁻²·s⁻¹·Pa⁻¹)</th>
<th>O₂P** (×10⁻¹³ mol·m⁻²·s⁻¹·Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyester</td>
<td>178</td>
<td>85</td>
<td>0.02</td>
<td>12</td>
</tr>
<tr>
<td>Polyvinyl chloride</td>
<td>93</td>
<td>30</td>
<td>1.2</td>
<td>12</td>
</tr>
<tr>
<td>Low-density polyethylene</td>
<td>13</td>
<td>500</td>
<td><strong>0.04–0.05</strong></td>
<td><strong>1005</strong></td>
</tr>
<tr>
<td>High-density polyethylene</td>
<td>26</td>
<td>500</td>
<td>8.9</td>
<td>224</td>
</tr>
<tr>
<td>Hydroxypropyl cellulose</td>
<td>15</td>
<td>33</td>
<td>6.2</td>
<td>300</td>
</tr>
<tr>
<td>Wheat gluten</td>
<td>3.3</td>
<td>192</td>
<td>5.1</td>
<td>1290</td>
</tr>
<tr>
<td>Soy protein</td>
<td>3.6</td>
<td>160</td>
<td>194</td>
<td>14</td>
</tr>
<tr>
<td>Corn zein</td>
<td>3.9</td>
<td>213</td>
<td>6.5</td>
<td>35</td>
</tr>
<tr>
<td>Fish myofibrillar protein</td>
<td>17</td>
<td>23</td>
<td>3.9–3.8</td>
<td>1–873***</td>
</tr>
</tbody>
</table>

* WVP (water-vapor permeability) (×10⁻¹² mol·m⁻²·s⁻¹·Pa⁻¹)
** O₂P (oxygen permeability) (×10⁻¹³ mol·m⁻²·s⁻¹·Pa⁻¹)
*** Measured under dry conditions, 873 measured under high relative humidity.

RT, Room Temperature
RH, Relative Humidity
Encapsulation Methods: Matrix Formation

Requirements: cost effective, scalable, gentle process

**Drip Capture**
- Med viscosity OK
- 400-1200 micron
- 0.5 Kg/nozzle/hr

**Spray Capture**
- 60 Kg/Nozzle/hr
- 50-200 microns
- Sterility and harvest difficulties
Encapsulation Methods: Core-Shell Coating

**Spray Drying**
- 60 Kg/Nozzle/hr
- 50-200 microns
- High shear and temp exposure

**Fluidized Bed Coating**
- High through put
- Double processing
- High humidity and temp exposure
ABN Encapsulation Technologies

1- Stabilize by High $T_g$ Materials

2- Protect with Polymeric Matrix
ABN’s Dehydration Process

Matrix Formation

Magnified Particles

Shredding

Dehydration

Milling
ABN Stabilized Probiotics: Storage Stability in Elevated Temperature (24-40°C) and Humidity (33% RH)

Shelf Storage Stability of *L. rhamnosus* at 40°C and 33% RH

- Free: **0.61 log loss/30 days**
- Encapsulated

Shelf Storage Stability of *L. paracasei* at 37°C and 33% RH

- Free: **0.65 log loss/90 days**
- Encapsulated
ABN Stabilized Probiotics: Storage Stability in Elevated Temperature (24-40°C) and Humidity (33% RH)
ABN Stabilized Probiotics: Gastric Stability (in a Gastric Juice Model)

Effect of *L. acidophilus* Concentration on Milk Acidifying Rate

![Graph showing the effect of *L. acidophilus* concentration on milk acidifying rate.]

2 hours incubation in simulated gastric juice at 37 °C and pH=1.2.

Probiotic Strain

<table>
<thead>
<tr>
<th>Probiotic Strain</th>
<th>Loss (log CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>Free</td>
</tr>
<tr>
<td>L2</td>
<td>Encapsulated</td>
</tr>
<tr>
<td>L3</td>
<td>Free</td>
</tr>
</tbody>
</table>
Survival of *Bifido* sp. in peanut butter spread (A_w 0.24) incubated at 25°C. Non-stabilized bacteria lost over 1 log CFU/g within 3 months, the stabilized bacteria survived for more than a year with a loss of 0.2 log CFU/g.
Survival of *Lactobacillus paracasei* in powder beverage (\(A_w \ 0.26\)) incubated in closed packaged at 25°C for 18 months. Non-stabilized bacteria lost over 4 log CFU/g within one month, the stabilized bacteria survived for 18 months with a loss of about 0.5 log CFU/g.
Summary

- **Claims**: Digestive health, Immune Support, Oral Health, Urogenital Health, Weight Management, etc.

- **Applications**: Supplements, Treats, Cereals, Beverages, Ice cream, Yogurt, Companion Animals.

- **Keys to Delivery**: Temperature, Humidity, Oxidation, Industrial process destruction, Gastric destruction, Targeted delivery.

- **Encapsulation Methods**: Freeze drying, Spray capture, Spray drying, Fluidized bed coating, preservation formulas.

- **Encapsulation Technologies**: High Tg Materials, Polymeric Matrixes, Controlled Dehydration.
Additional Information

• International Probiotics Association (IPA) news letters, events and general information: http://internationalprobiotics.org/


• European Food Safety Authority (EFA). Various publications on probiotics: http://www.efsa.europa.eu/

Publications


Thank you!