Yeast Biocapsules: More than just a carrier for food flavours

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INTRODUCTION

What has yeast ever done for us?
Properties of yeast
Target molecules – process improvements
Future prospects
What Has Yeast Ever Done For Us?

Over 5000 years ago yeast was used in fermentation for wine and bread-making.

Pure yeast used in brewing from 1883 by Carlsberg’s Emil Hansen.

Yeast cell factories.

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Good Yeast vs Bad Yeast

- Baker’s and Brewer’s yeast
  *Saccharomyces cerevisiae* or
  *S. pastorianus* (aka *S. carlsbergensis*)

- Food and drink spoilage caused by “wild yeast” and moulds.

- Yeast Infections (Candidiasis)
Biocapsules

Yeast cells as natural capsules for functional ingredients

Freeze-fracture cryo-SEM of active baker’s yeast (Saccharomyces cerevisiae)

Cell wall

Cell membrane
Why Use Yeast As An Inert Carrier?

- Pre-formed microcapsules
- Produces readily dispersible dry powders and granules
  - Spray drying or spray agglomeration
- Contents are protected by a robust cell wall
  - Products are amenable to blending, extrusion and high heat processing
- Ideal for small fat-soluble molecules (hydrophobes)
- Yeast is a readily available natural raw material, a commodity product with a consistent supply
1973
- Yeast used to absorb water soluble molecules
- Serozym Laboratories French patent 2179528

1977
- High lipid strains used for trapping hydrophobes
- Swift and Company US patent 40001480

1983
- Use of co-solvents to aid uptake
- Dunlop Ltd Patent EP 0085805

1986
- Encapsulation of pesticides
- Dunlop Ltd Patent GB 2162147
History - Process NPD

1987
- Aqueous mixing process using standard yeast strains
- AD2 Patent EP 0242135

1991
- Yeast biocapsules applied to textiles
- BTTG Patent EP 0511258

1991
- Process improvements in Japan

1991
- Yeast biocapsules used in pesticide formulations
- Welcome Foundation Ltd Patent WO2005030383
History - NPD

1993
- Process for de-odourising yeast biocapsules for laundry use
- Procter & Gamble Patent WO1993011869

1994
- Flavour containing microbes for tobacco materials
- Quest International Patent WO9409653

1998
- Passive diffusive uptake process characterised
- Bishop, et al. J. Microencapsulation 15, 761

2000
- Use in cosmetics
- Ciba Speciality Chemicals Patent EP 00810021

2000
- Use as a nicotine carrier for smoking cessation products
- Micap Ltd Patent EP 1176961
History - NPD

2004
- Use as a food flavour carrier adopted by Firmenich and launched as Thermarome® | Various patents followed

2005
- Baker’s yeast cell walls for agricultural use of terpenes
- Eden Research Plc Patent WO 2005113128

2005
- Herbicidal and antimicrobial compositions
- Micap plc Patents WO 2005102045 and WO 2005104842

2005
- Impact of water in release mechanism
- Normand, et al., J. Agric Food Chem. 53, 7532

2005
- Aerosol based cleaning products
- Reckitt Benkiser (UK) Ltd Patent WO2005030383
Target Ingredients

- **Flavours and tastes**
  - Flavour and aroma
  - Cooling agents
  - Health and wellness ingredients

- **Agriculture**
  - Fungicides
  - Insecticides and herbicides
  - Semiochemicals/attractants

- **Healthcare**
  - Pharmaceutical - APIs & Paracellular drug delivery
  - Repellents
  - Wound care, sanitizers, cleaning products
Same Old Challenges

- Convert liquids to solids - Improve handling
- Targeted delivery - Improve impact or bioavailability
- Controlled & delayed release - Process stability
- Masking taste and odour - bitter plant extracts and volatiles
- Protection for sensitive ingredients against:
  - UV
  - Heat
  - Moisture
  - Oxidation
- Isolation of reactive components
- Stabilisation of volatile ingredients to improve shelf life
  - Flavours and fragrances
Production - Spray Drying

- Emulsifiable liquids such as essential oils and liquid flavours
- Typically water or oil soluble principal components are typically mixed with a matrix material e.g. maltodextrin and gums or yeast
- A two-fluid nozzle disperses the liquid into fine droplets, water evaporates in the drying chamber forming dry particles
Production - Spray Drying

Cell wall
Cell membrane

30 µm Agglomerates @ 150-170 cells

Decreasing magnification
When yeast was used to encapsulate antimicrobial Tea Tree oil the initial rate of uptake was rapid over the first 30 minutes and approached a plateau after 5 hours.

Duckham et al. Proc. of 14th International Symposium on Microencapsulation, September 4-6 2003.
Localisation Within Cells

Confocal-fluorescence and scanning electron microscopy used to visualise encapsulation Nile red (in green) to indicate lipid droplets and DAPI stain to show the nucleus (in blue).
Mechanism Of Encapsulation

After Dardelle et al. (2007). Food Hydrocolloids. 21, 953-960
The structure of the yeast protective envelope comprises the cell wall and the lipid membrane, the key selective barriers in the uptake of fat soluble flavours.

*after Kilcher et al. (2008) Faraday Discuss. 139, 199-212*
Staining With Nile Red

- Nile Red is non-ionic and emission does not depend on local pH, or on the presence of specific chemical compounds.
- It has poor water solubility and exhibits bright red-yellow fluorescence in a hydrophobic environment.
- Exhibits solvatochromic behaviour.

NR ideal for targeting intracellular lipidic droplets

Greenspan et al. 1985
NR exhibits negligible fluorescence in water and fluoresces according to the medium it is in. Due to these useful spectral properties it has been extensively used for microscopic imaging purposes; in particular for intracellular lipidic droplets.
Yeast cells as microcapsules. Analytical tools and process variables in the encapsulation of hydrophobes in *S. cerevisiae*

Federica Ciamponi · Craig Duckham · Nicola Tirelli

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**Abstract** Yeast cells can be used as biocompatible and biodegradable containers for the microencapsulation of a variety of actives. Despite the wide application of this process, e.g. in the food industry, mechanism and controlling factors are yet poorly known. In this study we have studied kinetics and mechanistic aspects of the spontaneous internalization of terpenes (as model hydrophobic compounds) in *Saccharomyces cerevisiae*, quantifying their encapsulation through HPLC analysis and fluorescent staining of lipoidic bodies with Nile Red, while in parallel monitoring cell viability. Our results showed that this encapsulation process is essentially a phenomenon of passive diffusion with negligible relevance of active transport. Further, our evidence shows that the major determinant of the encapsulation kinetics is the solubility of the hydrophobe in the cell wall, which is inversely related to partition coefficient (log *P*).

**Keywords** Encapsulation · Yeast · Cell wall · Flavours · Diffusion
Hydrophobic Probes

Early studies showed that components of garlic oil were encapsulated with high efficiency.

Polymer probes were created to investigate compartmentalisation and test the molecular weight cut off characteristics of yeast biocapsules.

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Kilcher et al. (2007) Langmuir, 23 12309-12317
Fluorescent Probes Synthesised For The Study

The introduction of dansyl or 2-acetoxyethyl groups was accomplished by endcapping thiolate groups (for DA1100 to DA3800 at the termini of polysulfide chains) with dansyl acrylate and ethyl 2-bromoacetate, respectively.

The dansyl group was chosen for its high stability both to chemical agents and to photo-bleaching ($\lambda_{\text{exc}} = 343$ nm; $\lambda_{\text{em}} = 494$ nm)

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Kilcher et al. (2008) Faraday Discuss. 139, 199-212
Cell Membrane Removal

*S. cerevisiae* yeast cells initially in PBS, are transferred into DMSO for 2hr and then spun down and returned to PBS

A 20 nm thick gap appears where the lipid bilayer resided between the cytoplasm and cell wall, initially clearly visible, seems to disappear, as do the membranes of internal cell organelles. These membranes are not restored when cells are transferred back to water milieu after exposure to DMSO.

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*Kilcher et.al. (2008) Faraday Discuss. 139, 199-212*
Improvements In The Uptake Process

This shows how droplets of fluorescent polysulfides above 600 daltons could not pass across the cell wall using conventional aqueous mixing. Selected solvents in a water free system facilitated the process for very hydrophobic molecules to almost 4000 daltons.

Kilcher et.al. (2008) Faraday Discuss. 139, 199-212

Images courtesy of N. Tirelli, University of Manchester
Testing Yeast Viability During Encapsulation

Images of the localization and fluorescence of FUN-1 Cells were also stained 25 µM Calcofluor white to highlight the cell wall.
Raw Materials - Dead Yeast

- Yeast
Overview

**Trigger:**
- Moisture
- pH
- Temperature
- Pressure
- Shear

**Release:**
- Sustained Diffusion
- Burst
- Shear rupture

**Target:**
- Crop protection
  - Fungicide/Herbicide/Insecticide
- APIs
  - Oral/Topical/Paracellullar DD
- Flavours

[Diagram showing delayed release and sustained release with Core-Shell Capsule and Matrix Particle]
<table>
<thead>
<tr>
<th>Year</th>
<th>Development</th>
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<tbody>
<tr>
<td>2007</td>
<td>- Modelling of uptake and release in application&lt;br&gt;</td>
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<td>2007</td>
<td>- Flavoured tobacco products&lt;br&gt;</td>
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<tr>
<td></td>
<td>- Philip Morris Products S.A. Patent WO/2008/023271</td>
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<tr>
<td>2008</td>
<td>- Probing transport through cell walls&lt;br&gt;</td>
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<td>- Kilcher <em>et al.</em>, <em>Faraday Discuss.</em> <strong>139</strong>, 199</td>
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<tr>
<td>2009</td>
<td>- New Method of Encapsulation for larger hydrophobic molecules&lt;br&gt;</td>
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<td></td>
<td>- University of Manchester Patent WO 2009053711</td>
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<tr>
<td>2015</td>
<td>- Approved use for vine fungal pathogen control in Europe&lt;br&gt;</td>
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<td></td>
<td>- Eden Research Plc</td>
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BENEFITS: Improved Delivery

- Effective delivery
  - Modified flavour profiles
  - Improved bioavailability
  - A sustained release platform delivery system

- Protection from
  - Evaporation
  - High temperatures
  - High shear processes
  - Structurally - from pH changes and hydrolysis

- Prospects for new product concepts
- Stable aqueous dispersions for fat soluble ingredients
- Easy-to-handle: fine powder and liquid spray coatings
- Natural - retain ingredient labelling advantages
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