Enzymatic modification of phospholipids

Identification

Key words
Phospholipids (lecithin), emulsifiers, phospholipase, membrane structure

Latest version
2012/07/17

Completed by
DIL

How does it work?

Primary objective
Improved emulsifying properties, increase of dispersibility in aqueous systems or obtaining of nutritionally valuable phospholipids by enzymatic modification of phospholipids with phospholipases A₁, A₂, and D
Working principle

The principle is based on selective and specific Modification of phospholipid by enzymatic hydrolysis. Different enzymes can be used for the modification reactions.

**Phospholipase A₂ (EC 3.1.1.4) (PLA₂)** catalyses a cleavage of fatty acid at the sn2 position (Fig. 1) of the phospholipid molecule [1]. Lysophospholipids generated due to PLA₂ activity have better o/w emulsification properties than the corresponding natural phospholipids [2]. The HLB-value of the lysophospholipids increases with the hydrolysis degree and they become more hydrophilic. A hydrophilic phospholipid molecule can also be obtained by the phospholipase A₁ (EC 3.1.1.32) cleaving the fatty acid at position sn1. This application is used for degumming of edible oils during refining. Additionally, a free fatty acid is obtained from each phospholipid molecule [3].

**Phospholipase D (EC 3.1.4.4) (PLD)** catalyses the enzymatic cleavage of the phosphate ester bond of phospholipid molecule causing a release of the polar head group (Fig. 1). Additionally, PLD treatment can lead to transphosphatidylation with other polar groups, e.g. alcohols [4]. PLD is used for modification of polar head group of the phospholipid molecule in order to increase the content of particular phospholipid species, e.g. phosphatidylcholine (PC), phosphatidylserine (PS) or phosphatidic acid (PA), to alter their physicochemical and technological properties or to generate novel phospholipid derivates with modified head groups [2,5,6].

![Fig.1 Phospholipid molecule with cleavage locations of phospholipases A₁, A₂ and D. R₁,R₂: fatty acids residuals; R₃: alcohol](image)

**Additional effects**

- Increased stability of o/w emulsions which are prepared with lysophospholipids [7]
- Increased heat stability of emulsions prepared with PLA₂-and PLD-treated egg yolk [8,9]
- Better removal of lysophospholipids during degumming in the refining process of vegetable oil [2]
- Generating of nutraceuticals by application of PLD [5]
- Obtaining phospholipids with a high purity [3]

**Important process parameters**
- temperature, pH-value, reaction time, ion concentration, type and dosage enzyme, activities and side-activities of the phospholipases

**Important product parameters**
- water content

**What can it be used for?**

**Products**
- Lecithin blends, egg yolk, dairy products, bakery products, additives, vegetable oil, nutraceuticals, pharmaceuticals

**Operations**
- Structure forming, conversion, stabilizing
Solutions for shortcomings

Application of phospholipases follows the trend of using enzymes as an alternative to chemical processes. Modified phospholipids are used in foods, cosmetics and pharmaceuticals. Phospholipids with modified structure may be applied for producing nutraceuticals. Phosphatidylserine (PS) was shown to have positive effects on cognitive capacity and is used as nutritional health supplement for memory improvement. PS can be derived from soy lecithin which has been treated by PLD.

What can it NOT be used for?

Products

Products containing no additives because phospholipids must be declared (in EU as E322) [10]. For halal/kosher products, only lecithin modified by plant derived phospholipases can be used [8].

Operations

Restricted application of phospholipases due to source and product specificity of the enzymes.

Other limitations

Scale-up problems

Risks or hazards

Lecithin is regarded as a well-tolerated nontoxic compound. For enzymatic treatment solvent-free systems are preferred.

Implementation

Maturity

PLA₂ hydrolysis is available on industrial scale. Products are used in food, pharmaceuticals, plastics, coatings, cosmetics etc. However enzymatic modification of phospholipids by phospholipase D is only used in lab-scale.

Modularity /Implementation

In order to meet the specific needs of the food industry, the enzymes have to be permanently optimised by protein engineering, resulting in increased production costs.

Consumer aspects

Non-GMO lecithin should be used. Additionally, phospholipases originated from genetically modified microorganisms may be critical.

Legal aspects

The Codex Alimentarius Committee of the FAO/WHO has listed food-grade lecithins with recommended purity criteria for worldwide use. The EU-approved food additive number E322 comprise enzymatically hydrolyzed lecithins [10].

Environmental aspects

Compared to physical or chemical methods, the enzymatic approach allows a better control of the reactions, greatly reduces the consumption of toxic solvents, saves chemicals, energy and water due to mild reaction conditions, reduces waste and increases product yield [11].

Facilities that might be interesting for you

<table>
<thead>
<tr>
<th>Title</th>
<th>Institute/company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auditorium IRTA</td>
<td>IRTA</td>
</tr>
<tr>
<td>Clean room – Histocell</td>
<td>Noray</td>
</tr>
<tr>
<td>Video observation system for market research and product development tasks - Keki</td>
<td>NAIK EKI</td>
</tr>
</tbody>
</table>
Further Information

Institutes
DIL, TU München, Martin Luther Universität Halle Wittenberg, DTU Food, Texas A&M University

Companies
Lecithos Consulting, Lecipro Consulting, Unilever, Novozymes, Biocatalysts, Cargill

References
2. De Maria L.; Vind J.; Oxenboll K.M.; Svendsen A.; Patkar S. (2007), Phospholipases and their industrial applications. Applied Microbiology and Biotechnology 74 (2) 290-300

Source: