Natural Deep Eutectic Solvents as plasticizers and mechanical properties modifiers of edible films from dairy by-products

Andromachi Tzani, Dimitra Antonia Bagaki, Anastasia Detsi

Laboratory of Organic Chemistry, School of Chemical Engineering, National Technical University of Athens, Zografou Campus, 15780 Athens, Greece
The problem

Biopolymers

- The development of new bio-based plastics is of growing priority mainly due to health and environmental concerns derived by the petroleum-based plastics
- Among several types of natural polymers, protein-based films have emerged as potential bio-based plastics for producing biodegradable films and coatings.
- Edible films applied onto the surface of food in order to extend their self-life.

Milk proteins

- Several protein types can be obtained from milk: casein (80%) and whey proteins (20%) such as whey protein concentrate (WPC), isolate (WPI).
- Whey is a by-product from the cheese industry and is mainly considered to be a waste product.
- Whey proteins have been used as valuable food ingredients with high nutritional content and also as gelling, emulsifier and foam agents.

Novelty

- Use of industrial by-products, as starting material for film production considering them as valuable resources for the development on novel materials rather than wastes
- Design of novel protein biopolymers using green solvents as well as their extracts in order to improve their properties

Smart evaluation of dairy by-products in the development of added value products contributing in the “zero waste” goal.
Natural Deep Eutectic Solvents (NADES)

- Green Solvents of 21st century
- High stability
- Scalability
- Liquid phase in a wide temperature range
- Low cost
- Low vapor pressure
- Easy preparation
- Biodegradability
- Biocompatibility
- Tunable properties
- Multi-functionality

NADES

- Hydrogen Bond Donor (HBD)
- Hydrogen Bond Acceptor (HBA)

Deep Eutectic Solvent (DES)
Aim

1. Targeted design and synthesis of Natural Deep Eutectic Solvents (NADES)

2. NADES in the protein films formation process

3. Development of edible films for coating applications

Milk Proteins
- Casein Protein
- Whey Proteins

Properties
- Solubility
- Foaming
- Thickening
- Emulsification
- Gelation

Applications
- Surfactants
- Texture modifiers
- Foaming and gelling agents
- Thickening agents
- Emulsifiers

NADES
- Natural Deep Eutectic Solvents

Properties
- High Solvation activity
- Stabilizing Agents
- Protective Agents
- Bioactivity

Applications
- Extractions
- Treatment of raw materials/wastes
- Cosmetics
- Modification of desirable end characteristics
1. Targeted design and synthesis of Natural NADES

<table>
<thead>
<tr>
<th>NADES</th>
<th>Compound 1</th>
<th>Compound 2</th>
<th>Compound 3</th>
<th>Molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bet-Gly</td>
<td><img src="image1" alt="Compound 1" /></td>
<td><img src="image2" alt="Compound 2" /></td>
<td>-</td>
<td>1:2</td>
</tr>
<tr>
<td>Bet-Gly-W</td>
<td><img src="image1" alt="Compound 1" /></td>
<td><img src="image2" alt="Compound 2" /></td>
<td><img src="image3" alt="Compound 3" /></td>
<td>1:2:1</td>
</tr>
<tr>
<td>ChCl-Gly</td>
<td><img src="image1" alt="Compound 1" /></td>
<td><img src="image2" alt="Compound 2" /></td>
<td>-</td>
<td>1:2</td>
</tr>
<tr>
<td>ChCl-Gly-W</td>
<td><img src="image1" alt="Compound 1" /></td>
<td><img src="image2" alt="Compound 2" /></td>
<td><img src="image3" alt="Compound 3" /></td>
<td>1:2:1</td>
</tr>
<tr>
<td>Pro-Gly</td>
<td><img src="image1" alt="Compound 1" /></td>
<td><img src="image2" alt="Compound 2" /></td>
<td>-</td>
<td>1:2</td>
</tr>
<tr>
<td>NaOAc-Gly</td>
<td><img src="image1" alt="Compound 1" /></td>
<td><img src="image2" alt="Compound 2" /></td>
<td>-</td>
<td>1:9</td>
</tr>
</tbody>
</table>

Wang et. al. (2016).<sup>1</sup>

https://doi.org/10.1016/j.talanta.2016.01.042

- Plasticizing effect (replacement of conventionally used additives)
- Alteration of properties (e.g. elasticity, water vapor permeability)
- Zero waste process
- Added-value protein films
2i. NADES in the protein films formation process

1. Targeted design and synthesis of Natural Deep Eutectic Solvents (NADES)

2. NADES in the protein films formation process

3. Development of edible films for coating applications

- Edible films
- Bioplastics for packaging and coatings

Proteins from various sources

Whey Protein Isolate

Packaging

Biodegradation

Biopolymers
2ii. NADES in the protein films formation process

- **Protein addition in warm water**
- **Ph measuring**
- **NaOH addition**
- **pH measuring**
- **Water bath heating**
  - **water bath heating / protein dissolution**
  - **NaDES addition as plasticizer and properties modifier**

**Texture analysis**
- Burst Strength
- Elasticity
- Cohesiveness
- Adhesiveness

**Dissolution in water**

**Water-vapor permeability**

**Thickness**

**Weight**

**Film formation**
- **Casein film**
- **WPI film**

**Casting method**
Casein-based films using NADES as plasticizers

Various Casein/plasticizer ratio (w/w) were tested in all cases: 3:1, 2:1, 1:1, 1:2.

<table>
<thead>
<tr>
<th>Casein / plasticizer ratio (w/w)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bet/Gly 1:2 2:1</td>
<td>✔</td>
</tr>
<tr>
<td>Bet/Gly 1:2 1:1</td>
<td>✔</td>
</tr>
<tr>
<td>Bet/Gly/W 1:2:1 2:1</td>
<td>✔</td>
</tr>
<tr>
<td>Bet/Gly/W 1:2:1 1:1</td>
<td>✔</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Casein / plasticizer ratio (w/w)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChCl/Gly 1:2 2:1</td>
<td>✔</td>
</tr>
<tr>
<td>ChCl/Gly/W 1:2:1 2:1</td>
<td>✔</td>
</tr>
<tr>
<td>L-Pro/Gly 1:2 1:1</td>
<td>✔</td>
</tr>
<tr>
<td>SA/Gly 1:9 1:1</td>
<td>✔</td>
</tr>
</tbody>
</table>

Chamomile Extract
Bet/Gly/W (1:2:1)

Result

1:1
Various Casein/plasticizer ratio (w/w) were tested in all cases: 3:1, 2:1, 1:1, 1:2.

<table>
<thead>
<tr>
<th>Casein / physical mixtures ratio (w/w)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical mixture Betaine – Glycerol (1:2)</td>
<td>✔</td>
</tr>
<tr>
<td>Physical mixture Betaine – Glycerol - Water (1:2:1)</td>
<td>✔</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Casein / physical mixtures ratio (w/w)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical mixture L-Proline – Glycerol (1:2)</td>
<td>✔</td>
</tr>
<tr>
<td>Physical mixture Betaine – Glycerol - Water (1:2:1)</td>
<td>✔</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Casein/ Glycerol ratio (w/w)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:1</td>
<td>✗</td>
</tr>
<tr>
<td>1:1</td>
<td>✗</td>
</tr>
<tr>
<td>1:2</td>
<td>✗</td>
</tr>
</tbody>
</table>
Various WPI/plasticizer ratio (w/w) were tested in all cases: **1.4:1, 1.3:1, 1:1, 1:1.5, 1:2.**

<table>
<thead>
<tr>
<th>WPI / plasticizer ratio (w/w)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bet/Gly (1:2)</td>
<td>✔</td>
</tr>
<tr>
<td>1:1</td>
<td>✔</td>
</tr>
<tr>
<td>Bet/Gly/W (1:2:1)</td>
<td>✔</td>
</tr>
<tr>
<td>1:1</td>
<td>✔</td>
</tr>
<tr>
<td>Pro/Gly (1:1)</td>
<td>✔</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>WPI / Glycerol ratio (w/w)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol (Reference)</td>
<td>✘</td>
</tr>
<tr>
<td>1:1</td>
<td>✘</td>
</tr>
</tbody>
</table>
Films Characterization

- **Water Vapor Permeability**:
  - L-Pro-Gly (1:2) in casein/NADES ratio=1:1
  - Bet-Gly-W (1:2:1) in casein/NADES ratio=1:1

- **Burst Strength**:
  - Casein/NADES 1:1
  - Casein/NADES 1:2
  - Casein/NADES 1:3

- **Casein**: L-Pro-Gly (1:2) in casein/NADES ratio=1:1
- **WPI**: Bet-Gly-W (1:2:1) in casein/NADES ratio=1:1

Extract -> lower WPI than only NADES as plastisizer
Films Characterization (ii)

Cohesiveness

Adhesiveness

Elasticity
Films Characterization (ii)

## Preliminary Results

<table>
<thead>
<tr>
<th>Coating</th>
<th>Carrot</th>
<th>Cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without (A)</td>
<td>Commercial Membrane (B)</td>
</tr>
<tr>
<td>Weight Loss (%) at 25 °C</td>
<td>88.264</td>
<td>48.694</td>
</tr>
<tr>
<td>Weight Loss (%) at 30 °C</td>
<td>81.3891</td>
<td>83.5337</td>
</tr>
</tbody>
</table>

### Ongoing study...
- WPI-based films as coatings
- Alteration on the coating procedure
- Bioactivity measurements on the protein films
Development of added-value products from the dairy industry wastes: Utilization of whey cheese using biocompatible green solvents

Consulting support from the company FrieslandCampina Hellas-NOUNOU
Thank you for your attention

Questions