Development of Sustainable Protein Hydrolysates from Okara and Its Application in Food and Sustainable Packaging

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A. Introduction

- ✓ The antioxidant properties of soy dregs or okara protein hydrolysates have been ascribed to the cooperative effect of several properties including their ability to scavenge free radicals and other antioxidative effect.

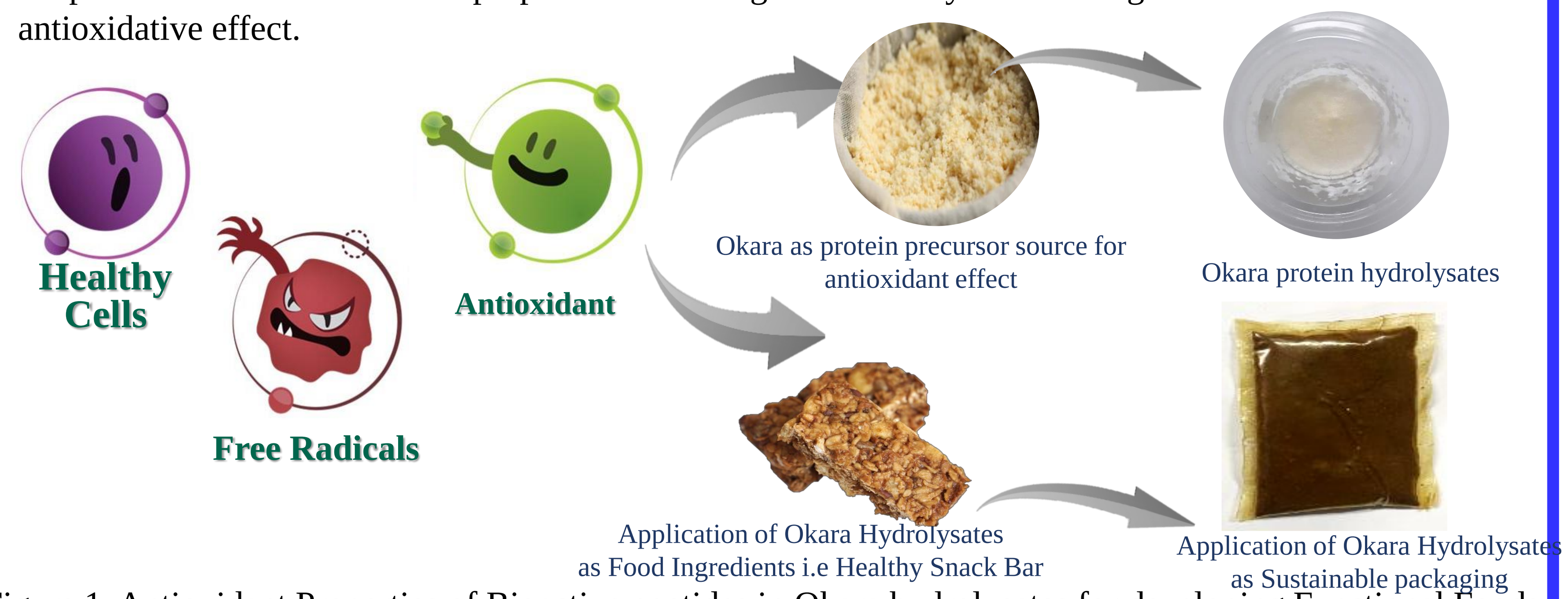


Figure 1. Antioxidant Properties of Bioactive peptides in Okara hydrolysates for developing Functional Foods and Sustainable Packaging

- ✓ Approximately almost 10% of the world's soybean production is used directly for human food [1]. Okara hydrolysates has several bioactive compounds for developing ingredients in developing functional foods and also for development of sustainable packaging such as edible coating and edible films. Okara has a high nutritive value due to its high-quality protein, fat, carbohydrates, fiber, vitamin, and minerals that are still can be utilized further [2]. Hydrolysis okara using green approach such as enzymatic reactions can results several bioactive peptides that has functional properties such as antioxidant activities.
- ✓ *In silico* analysis is one approach of the common types of bioinformatics that is widely used in analyzing the amino acid sequence, protein domain, and protein structure, through a computational approach [3,4].

B. Aims

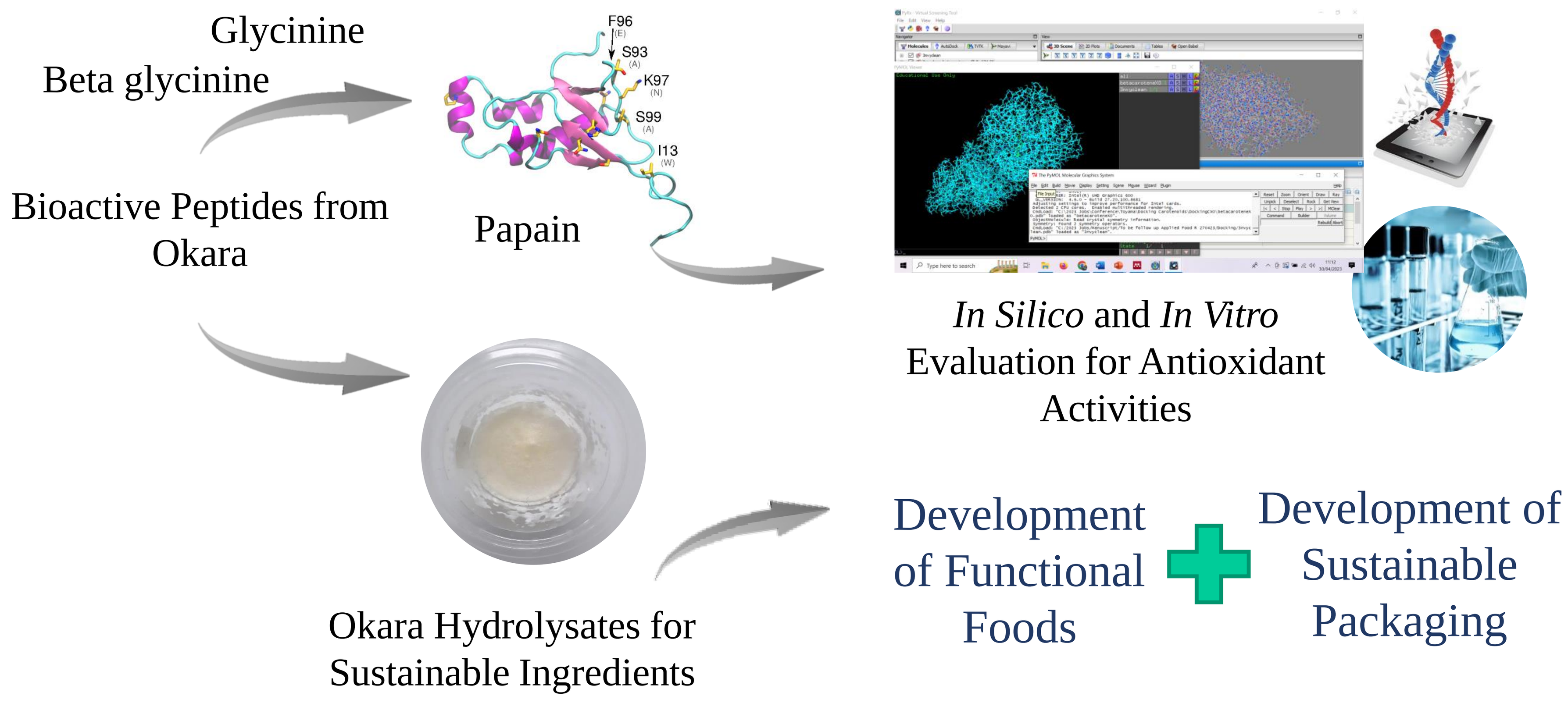


Figure 2. Aims of the research

- ✓ The objective of this study was to investigate the characterization of okara hydrolysates using *in silico* and *in vitro* assays and its application for the development of functional food and sustainable packaging

C. Methods

Analysis:

1. Protein sequence (UniProt)
2. Antioxidant activity prediction (BIOPEP)
3. Peptide score, sensory evaluation and physicochemical evaluation (Peptide Ranker)
4. Toxicity Prediction (ToxinPred)
5. Molecular docking of ligand (peptide) against XO (Xanthine Oxidase) and MPO (Myeloperoxidases)

Step 1 : *In silico* bioactive peptide analysis from okara protein using papain enzyme

Enzyme Simulation:

1. Papain (EC 3.4.22.2),
2. Docking against XO (Xanthine Oxidase) and MPO (Myeloperoxidases)

Analysis

1. Evaluation of Antioxidant Acttivies using *in vitro* approach

Step 2: *In vitro* bioactive peptide analysis from okara protein using papain enzyme

***In vitro* analysis:**

1. DPPH scavenging activities
2. FRAP method

Analysis

1. Characterization of Functional Food (Physicochemical and Sensory Evaluation)
2. Characterization of Sustainable Packaging (Edible Film and Edible Coating)

Step 3: Development of Functional Foods and Sustainable Packaging (Edible Coating and Edible Film)

Characterization of Product

1. Physicochemical characteristic of functional food
2. Sensory evaluation of functional food
3. Sustainable packaging evaluation

D. Results and Discussion

Step 1 : *in silico* bioactive peptide analysis from okara protein using papain enzyme

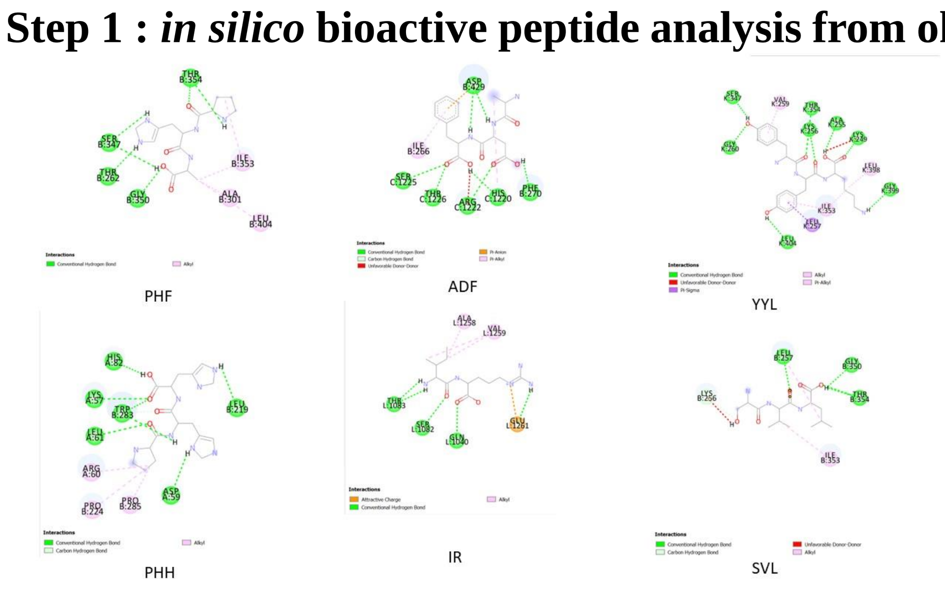


Figure 3. 2D Interaction ligands (bioactive peptides) and XO

- ✓ Several antioxidant bioactive peptides from the okara protein hydrolysates were evaluated using in silico approach e.g. Proline-Hystidine-Phenylalanine (PHF), Alanine-Aspartic Acid-Phenylalanine (ADF), Tyrosine-Tyrosine-Leucine (YYL), Proline-Hysitidine-Hystidine (PHH), Isoleucine-Arginine (IR), Serine-Valine-Leucine (SVL). Further explanation of results are available in Published Journal [3,4]

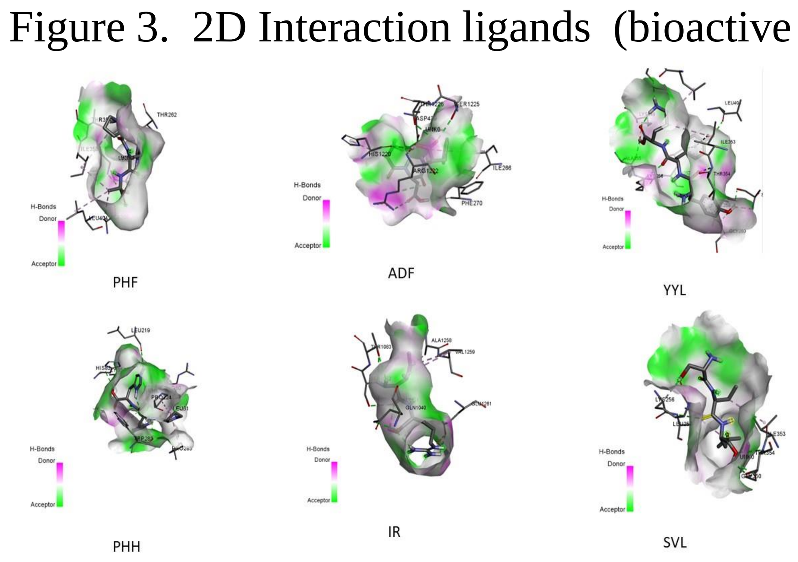
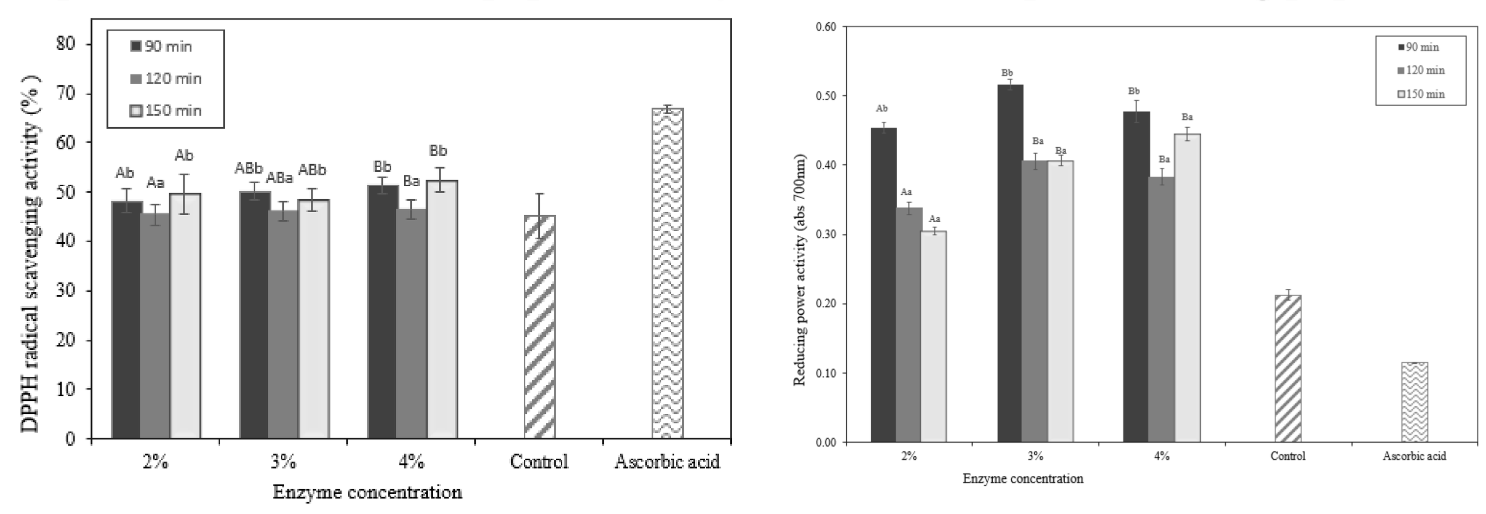


Figure 4. 3D Interaction ligands (bioactive peptides) and XO

- ✓ However, as shown in **Figure 3 and Figure 4** several interactions of ligands (bioactive peptides) to the protein can be seen in 2D and 3D.
- ✓ Further explanation of results are available in Published Journal [3,4]

Step 2 : *In vitro* bioactive peptide analysis from okara protein using papain enzyme



A-BDifferent capital letters indicate statistical difference between enzyme concentration (p<0.05).
a-bDifferent non capital letters indicate statistical difference between time of hydrolysis (p< 0.05).
Values are mean ± standard errors.

Figure 5. DPPH Radical Scavenging and FRAP Analysis of Okara Hydrolysate

- ✓ We also found that the hydrolysate of okara protein with the addition of 4% enzyme treatment of papain and hydrolysis time of 120 minutes had the highest yield and also the degree of hydrolysis.

D. Results and Discussion

Step 3.1 : Development of Functional Foods

Figure 6. Several functional food developed from okara hydrolysates

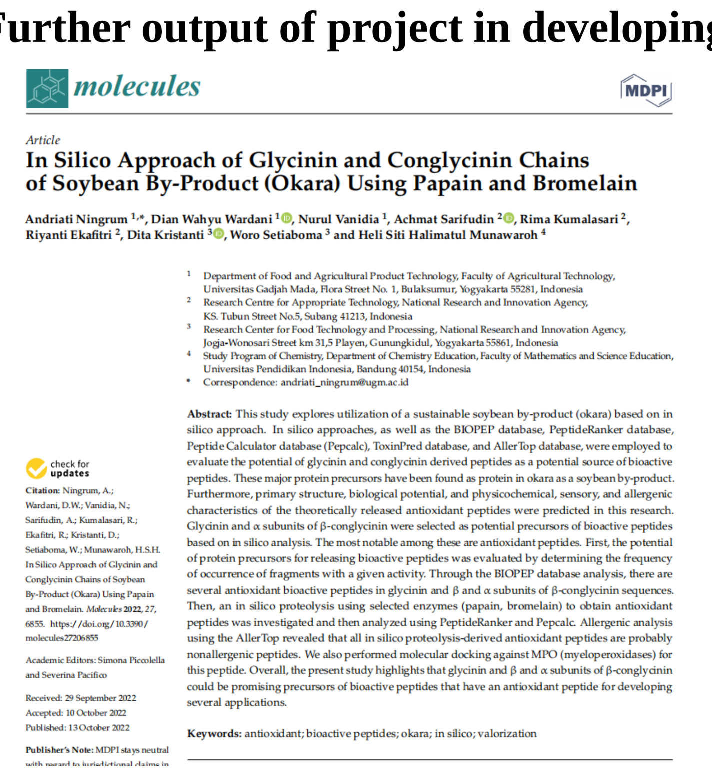
- ✓ We also found that the hydrolysate of okara protein can be added as bioactive ingredients in smoothies, healthy snack bar and also non gluten cookies (Figure 6)
- ✓ The functional products has higher antioxidant activities (rather that control/without addition of okara hydrolysates) and better sensory acceptance by panelist

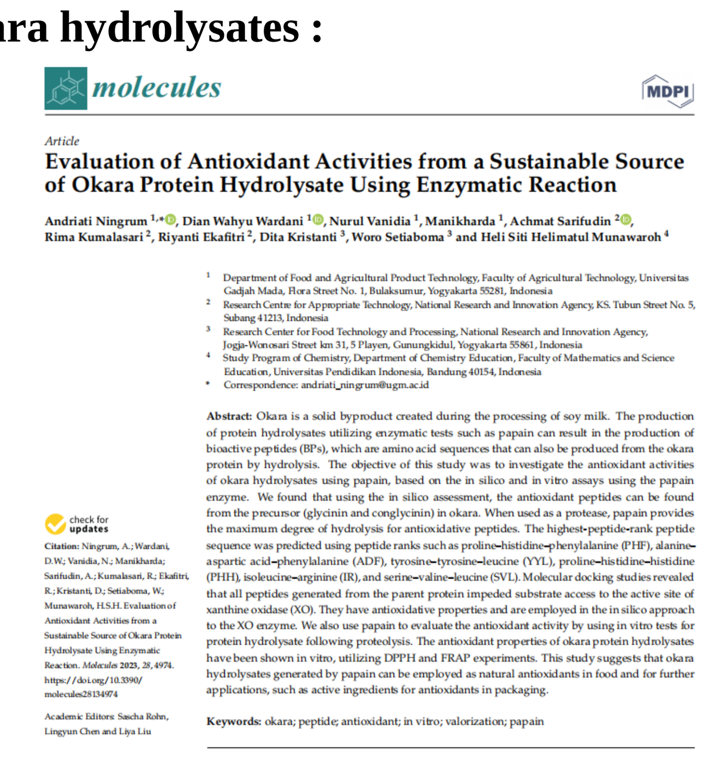
Step 3.2 : Development of Sustainable Packaging

Figure 7. Several sustainable packaging from okara hydrolysates

- ✓ We also found that the hydrolysate of okara protein can be added as bioactive ingredients in developing sustainable packaging such as edible coating and film (Figure 7)
- ✓ The edible film from okara protein hydrolysates has a more protective effect on instant coffee during storage. On the other hand, the edible coating from okara protein hydrolysates is also implemented to maintain the quality of minimally processed papaya.

Further output of project in developing okara hydrolysates :





E. Conclusions

- ✓ Based on the *in silico* assay, the protein precursor in okara such as glycinin and conglycinin may generate several bioactive peptides with an antioxidant effect.
- ✓ The molecular docking of okara hydrolysates generated by the papain enzyme successfully can be performed using molecular docking to XO and MPO as the enzyme that contributes to antioxidant activity.
- ✓ On the other hand, the okara hydrolysate also has potential properties for further application in food matrices such as natural antioxidant agents and can be incorporated in developing functional food and sustainable active packaging such as edible coating and film

F. Acknowledgment

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Reference

1. Colletti, A.; Attrovio, A.; Boffa, L.; Mantegna, S.; Cravotto, G. Valorisation of by-products from soybean (Glycine max (L.) Merr.) processing. *Molecules* 2020, *25*, 1–33, doi:10.3390/molecules25092129.
2. Sbroggio, M.F.; Montilha, M.S.; Figueiredo, V.R.G. de; Georgetti, S.R.; Kurozawa, L.E. Influence of the degree of hydrolysis and type of enzyme on antioxidant activity of okara protein hydrolysates. *Food Sci. Technol.* 2016, *36*, 375–381, doi:10.1590/1678-457X.000216.
3. Ningrum, A.; Wardani, D.W.; Vanidia, N.; Sarifudin, A.; Kumalasari, R.; Ekafitri, R.; Kristanti, D.; Setiaboma, W.; Munawaroh, H.S.H. In Silico Approach of Glycinin and Conglycinin Chains of Soybean By-Product (Okara) Using Papain and Bromelain. *Molecules* 2022, *27*, 1–11, doi:10.3390/molecules27206855.
4. Ningrum, A.; Wardani, D.W.; Vanidia, N.; Manikharda; Sarifudin, A.; Kumalasari, R.; Ekafitri, R.; Kristanti, D.; Setiaboma, W.; Munawaroh, H.S.H. Evaluation of Antioxidant Activities from a Sustainable Source of Okara Protein Hydrolysate Using Enzymatic Reaction. *Molecules* 2023, *28*, 4974, doi:10.3390/molecules28134974.