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. Okara as protein precursor source for Okara protein hydrolysates Healthy antioxidant effect Antioxidant **Cells Free Radicals** Application of Okara Hydrolysates Application of Okara Hydrolysate as Food Ingredients i.e Healthy Snack Bar as Sustainable packaging

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].

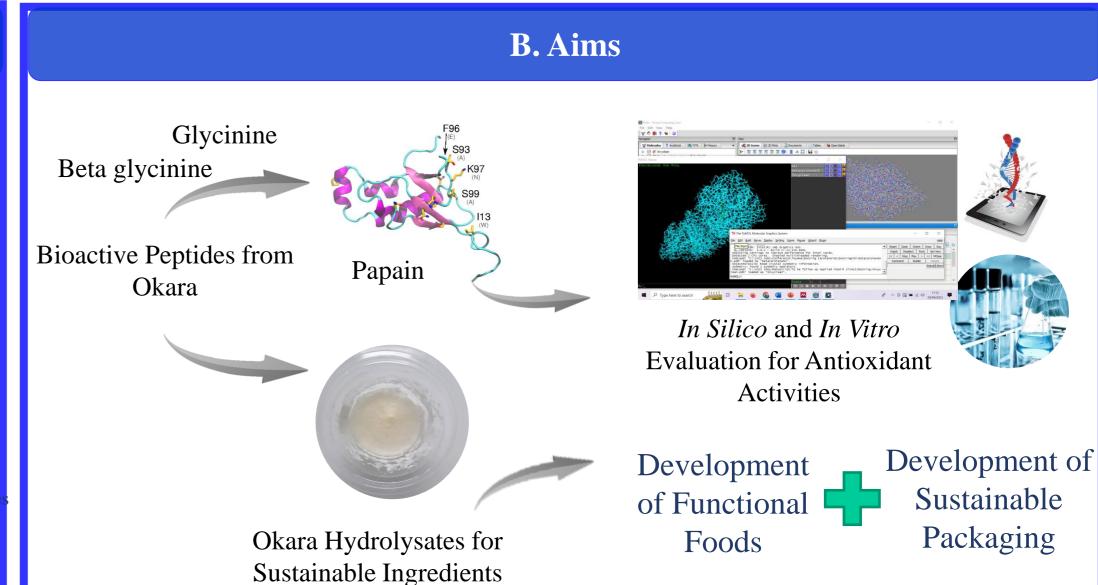


Figure 2. Aims of the research

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

Protein sequence (UniProt)

Analysis:

- Antioxidant activity prediction (BIOPEP)
- Peptide score, sensory evaluation and physicochemical evaluation (Peptide Ranker) Toxicity Prediction (ToxinPred)
- 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),
- Docking against XO (Xanthine Oxidase) and MPO (Myeloperoxidases)

- **Analysis** Analysis
- 1. Characterization of Functional 1. Evaluation of Antioxidant Food (Physicochemical and Sensory Acttivies using in vitro approach 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)

In vitro analysis:

1. DPPH scavenging activities 2. FRAP method

Step 2: In vitro bioactive

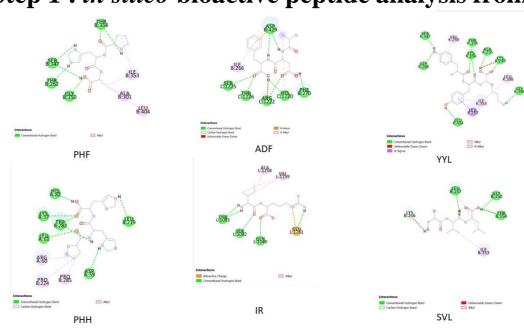
peptide analysis from okara

protein using papain enzyme

- **Characterization of Product** Physicochemical characteristic of functional food
- Sensory evaluation of functional food -Sustainable packaging evaluation

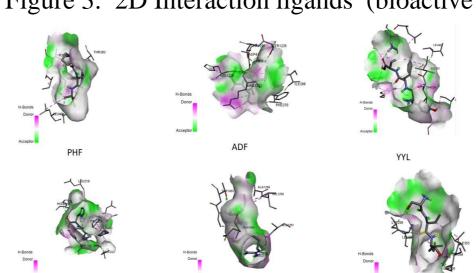
D. Results and Discussion

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



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

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

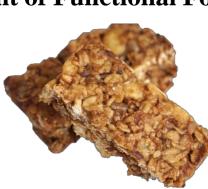


- ✓ However, as shown in Figure 3 and Figure 4 several interactions of ligands (bioactive peptides) to the protein can be seen in 2D and 3D.
- ✓ Further explanation of results are available in Published Journal [3,4]

D. Results and Discussion

Step 3.1: Development of Functional Foods





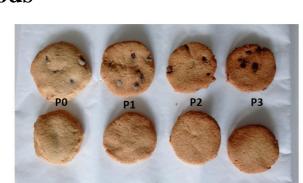


Figure 6. Several functional food developed from okara hydrolysates

- ✓ 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





- ✓ We also found that the hydrolysate of okara protein can be added as bioactive ingredients in developing
- ✓ 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

Figure 7. Several sustainable packaging from okara hydrolysates

- sustainable packaging such as edible coating and film (Figure 7)
- maintain the quality of minimally processed papaya.

molecules In Silico Approach of Glycinin and Conglycinin Chains of Soybean By-Product (Okara) Using Papain and Bromelain Andriati Ningrum ^{1,}*, Dian Wahyu Wardani ¹0, Nurul Vanidia ¹, Achmat Sarifudin ²0, Rima Kumalasari ², Rivanti Ekafitri², Dita Kristanti³0, Woro Setiaboma³ and Heli Siti Halimatul Munawaroh Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Hora Street No. 1, Bulaksumur, Yogyakarta 55281, Indonesia Research Centre for Appropriate Technology, National Research and Innovation Agency, KS. Tubun Street No.5, Subang 4213, Indonesia Research Center for Food Technology and Processing, National Research and Innovation Agency, Jogia-Wonosai Street km 31,5 Palyen, Gumungkidul, Yogyakarta 55861, Indonesia Study Program of Chemistry, Department of Chemistry Education, Faculty of Mathematics and Science Education Universities Pand-Iddian Jodgonesia, Bandung (1015, Hadonesia) Abstract: This study explores utilization of a sustainable soybean by-product (okara) based on in silico approach. In silico approaches, as well as the BIOPEP database, PeptideRanker database, Peptide Calculator database (Pepcalc), ToxinPred database, and AllerTop database, were employed to evaluate the potential of glycinin and conglycinin derived peptides as a potential source of bioactive Citation: Ningrum, A.; Wardani, D.W.; Vanidia, N.; Sarifudin, A.; Kumalasari, R.; Furthermore, primary structure, biological potential, and physicochemical, sensory, and allergenic characteristics of the theoretically released antioxidant peptides were predicted in this research. Glycinin and α subunits of β -conglycinin were selected as potential precursors of bioactive peptides Ekafitri, R.; Kristanti, D.; based on in silico analysis. The most notable among these are antioxidant peptides. First, the potential Setiaboma, W.; Munawaroh, H.S.H. In Silico Approach of Glycinin and Conglycinin Chains of Soybean By-Product (Okara) Using Papain of protein precursors for releasing bioactive peptides was evaluated by determining the frequency of occurrence of fragments with a given activity. Through the BIOPEP database analysis, there are several antioxidant bioactive peptides in glycinin and β and α subunits of β -conglycinin sequences. Then, an in silico proteolysis using selected enzymes (papain, bromelain) to obtain antioxidant and Bromelain. Molecules 2022, 27

peptides was investigated and then analyzed using PeptideRanker and Pepcalc. Allergenic analysis

sing the AllerTop revealed that all in silico proteolysis-derived antioxidant peptides are probably

nonallergenic peptides. We also performed molecular docking against MPO (myeloperoxidases) for

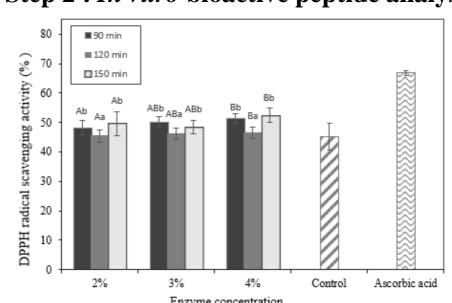
this peptide. Overall, the present study highlights that glycinin and β and α subunits of β -conglycinin could be promising precursors of bioactive peptides that have an antioxidant peptide for developing

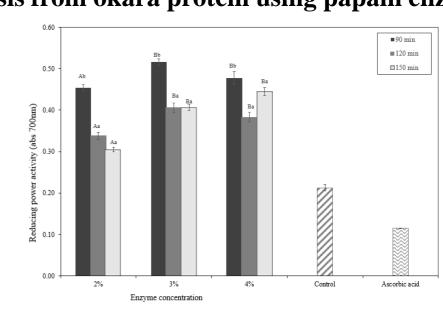
 $\textbf{Keywords:} \ antioxidant; bioactive \ peptides; okara; in \ silico; valorization$



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

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





- A-B Different 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).

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.

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 antioxidative 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

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- 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.
- 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 um, 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 Bromelain. Molecules 2022, 27, 1–11, doi:10.3390/molecules27206855.
- 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.