A review of protein hydrolysis fermented foods and their potential for health benefits

V P Yarlina, M Djali and R Andoyo
Faculty of Agricultural Industrial Technology, Universitas Padjadjaran, Indonesia

Email: vira.putri.yarlina@unpad.ac.id

Abstract. Fermented foods are consumed in many countries and potential benefits of health. Fermented products using microorganisms resulting in different type of product. The advantages of fermented foods are easy to digest, one of them is Protein. Protein composition changed during fermentation. Protein hydrolysis by enzymatic hydrolysis of whole protein sources by appropriate proteolytic enzymes under controlled conditions. This review article is focused on the process of protein hydrolysis in fermented foods, quantitative and qualitative analysis protein hydrolysis by various methods and specific protein hydrolysis having benefits of health. Some fermented food products such as soy fermentation that gets protein hydrolyzate are Glycinin and β-conglycinin. Some of the bioactive peptides in soybean fermentation are used for health, such as antioxidant, antimicrobial, anti-diabetic and anti-cancer activities. Many other fermented food products can be explored and use this information to develop fermented food products.

1. Introduction
Fermentation is one of the biologically processes that use microorganisms. The fermentation process can spontaneous or unspontaneous. Fermentation aims to extend shelf life and improve the quality of these foods. Fermented foods have a positive impact on health, especially increasing the nutritional value, so fermented foods are called functional foods [1].

The fermented based on milk, vegetables, fruits, meat and fish, bean and cereals. Yoghurt (fermented milk), Natto (fermented soybean), Kimchi (fermented cabbage), Sauerkreut (fermented cabbage), Sayur Asin, Meju, and Tempeh (fermented soybean) were generally popular in Asian countries, especially China, Japan, Korea, Indonesia, and Philippines. Generally, The fermentation process in Asian countries is spontaneous fermentation. Spontaneous fermentation was employed unknowingly, without any scientific knowledge of the fermentation process based on the origin of the food, raw materials, processing, and even depending on the person who prepares the food.

Fermentation processes are broken down complex organic compounds into smaller molecules by the microorganism, which exert various physiological functions beyond their nutritional properties. During fermentation, microorganisms produces enzyme such as amylase, protease and lipases hydrolyze the polysaccharides, proteins, and lipids to non-toxic products and rich nutritional value. Protease is proteolysis enzyme release of peptides with 2–20 amino acids and their activity depends on chain length, amino acid composition and sequence of amino acids. Protein hydrolysates are defined as a complex mixture of oligopeptides, peptides, and free amino acids that are produced by partial or extensive hydrolysis, while biopeptides or bioactive peptides are defined as peptides that possess beneficial pharmacological properties [1].
Protein hydrolysates from fermented food addition nutritional and pharmacological properties such as antioxidant [2], antimicrobial [3], anticancer [4], ACE-inhibitory, and antidiabetic [5]. Besides that, protein hydrolysate improves the digestibility, it can be utilized by the body to increase growth and body weight. Protein hydrolysate identified functional value by amino acid sequencing. This review article is focused on the process of protein hydrolysis in fermented foods, quantitative and qualitative analysis protein hydrolysis by various methods and specific protein hydrolysis having benefits of health.

2. Materials and methods

2.1. Materials
The material used in this journal is secondary data sources and publications from various quality international scientific journals. These international data sources and publications are used for the preparation of bioactive peptides so that potential health benefits.

2.2. Methods

2.2.1. Peptide library. The preparation of the peptide library is done by searching the library of related journals. Journals obtained from reputed journals that are known to be peptide sequences so known the health benefits.

2.2.2. Compilation of peptide analysis libraries. Preparation of analysis methods to determine amino acid sequences and peptide analysis based on literature obtained from reputable journals. The analysis methods used include SDS-PAGE, RP-HPLC, and LC-ESI-MS.

2.2.3. Bioactive peptide produced potential health benefits. The preparation of bioactive peptides can be predicted by using BioInformatics literature.

3. Result and discussion
Fermentation is biologically processed to produce peptides with microorganisms. Peptides are obtained by the presence of hydrolysis using proteolytic enzyme of protein and during food processing (cooking, fermentation, and ripening). The resulting peptide is called the bioactive peptide is known for its protein-inhibitory ability interactions to small size and specificity. In the last decade, bioactive peptides are known to have benefits in increasing the health.

The fermentation process uses bacteria and fungi. Generally, bacterial fermentation uses lactic acid bacteria (LAB) in the presence of proteolytic enzymes that can produce bioactive peptide. In addition, fermentation using fungi such as Aspergillus oryzae and Aspergillus sojae have a long tradition of safe use in the production of fermented foods [6]. In addition to the fermentation process, protease catalyze the hydrolysis of peptide bonds in proteins with enzymatic hydrolysis. Schematic representation of productions of bioactive peptides[7], [8].

Peptides are amino acid chains that are interwoven in the presence of peptide bonds. The number of amino acids in the peptide is below 50 molecules, but if more than 50 molecules are called proteins. Peptides have lower molecular weight and less secondary structures, as well as a higher number of ionizable groups and exposure of hydrophobic groups than the native protein. Amino acid sequences expressed as bioactive peptides such as amino acids with aromatic and aliphatic groups such as Pro, Phe, and Tyr on C-terminal atoms and Val and Ile at N-terminal positions are thought to have a role as ACE (angiotensin I converting enzyme) or antihypertension [9].

Bioactive peptide compound antihypertensive such as Tyr-Pro-Tyr-Tyr [10], Val-Pro-Pro; Ile-Pro-Pro [11], Gly-Tyr; Ala-Phe; Val-Pro; Ala-Ile; Val-Gly [12], Leu-Ile Val-Thr-Gln [13], Val-Pro-Pro, Iso-Pro-Pro, Gly-Tyr, Ala-Phe, Val-Pro, Ala-Ile, dan Val-Gly [14], Ala-Trp, Ala-Tyr, Gly-Trp dan Ser-Tyr [1], Glu-Val-Ser-Leu-Asn-Ser-Gly-Tyr-Tyr, Pro-Gly-Thr-Ala-Val-Phe-Lys dan Val-His-Leu-Pro-Pro [15], Arg-Pro-Lys-His-Pro-Ile-Lys-His-Gln-Gly-Leu-Pro-Gln-Glu-Val, Glu-Val-Leu-Asn-Glu-Asn-
Leu-Leu-Arg-Phe, Phe-Val-Ala-Pro-Phe-Pro-Glu-Val-Phe-Gly-Lys, Tyr-Gln-Glu-Pro-Val-Leu-Gly-Pro-Val-Arg-Gly-Pro-Phe, Tyr-Gln-Glu-Pro-Val-Leu-Gly-Pro-Arg-Gly-Pro-Phe-Pro-Ile dan Tyr-Gln-Glu-Pro-Val-Leu-Gly-Pro-Arg-Phe-Pro-Ile-Ile-Val [16].

Bioactive peptide compound antioxidant such as Leu-His-Tyr, Leu-Ala-Arg-Leu, Gly-Gly-Glu, Gly-Ala-His, Gly-Ala-Trp-Ala, Pro-His-Tyr-Leu and Gly-Ala-Leu-Ala-Ala-His [17], Ile-Arg-His-Phe-Asn-Glu-Gly-Asp-Val-Leu-Val-Ile-Pro-Gly-Pro-Val-Pro-Tyr, Ile-Arg-His-Phe-Asn-Glu-Asp-Val-Leu-Val-Ile-Pro-Arg-Val-Pro-Tyr, Tyr-Ala-Arg-Leu-Ser-Glu-Gln-Asp-Ile-Phe-Pro-Arg-Pro, Ile-Tyr-Arg-Phe-Glu-Gly-Asp-Leu-Ile-Pro-Ala-Val, and Val-Ser-Ile-Ile-Asp-Thr-Asn-Ser-Leu-Glu-Asn-Gln-Leu-Asp-Gln-Met-Arg-Arg [18].

Bioactive peptide in fermented food have many nutritional benefits. Fermented food with Lactic Acid Bacteria and fungi. Fermented cow’s milk with Lactobacillus delbrueckii subsp. Bulgaricus LA 2. Soybean fermentation with Bacillus spp. in Natto product. Fungi fermentation several fermented with soybean. In Indonesia, tempeh product one of fermentation Rhizopus sp. fungi with soybean.

**Figure 1.** Bioactive peptides.

Determine the peptide analysis method such as SDS-PAGE, RP-HPLC, Electrospray ionization mass spectrometry (ESI/MS). Mechanism SDS-PAGE is peptide migration depends both on their molecular mass and on their net charge at the electrophoresis pH [21]. Polyacrylamide gel electrophoresis (PAGE) technique are continues to lay a major role in protein purification and in protein/protein and protein/small ligand interaction.

RP-HPLC (reversed-phase high-pressure liquid chromatography) methods employed in proteomics. The mechanism of RP-HPLC is able to separate polypeptide of nearly identical sequences and trypsin digestion. RP-HPLC separates polypeptides based on subtle differences in the “hydrophobic foot” of the polypeptide being separated [21]. Sensitive RP-HPLC to protein conformation results in the separation of polypeptides.

Electrospray ionization mass spectrometry (ESI/MS) is important techniq the clinical laboratory for structural study or quantitative measurement of metabolites in a complex biological sample [22].

RP-HPLC is compatible with electrospray ionization mass spectrometry (ESI/MS), allowing these techniques to be used in combination for analysis peptides and protein digests. RP-HPLC enhanced separation capabilities, higher sensitivity, and acquisition of molecular information from multiple tandem mass spectrometric experiments related to the structure of the peptides [23].

Product fermentation with bioactive peptide and the potential for benefits of health in shown table 1.
Table 1. Bioactive peptide and bioactivity potential for benefits of health.

| Source            | Microorganism                  | Peptides                      | Bioactivity          | Reference |
|-------------------|--------------------------------|-------------------------------|----------------------|-----------|
| Cow’s Milk Fermentation | Lactobacillus delbrueckii subsp. Bulgaricus LA 2 | Tyr-Gly-Leu-Phe | ACE-inhibition | [19]      |
|                   |                                | Lys-Cys-Arg-Arg-Trp-Glu-Trp-Arg-Met-Lys-Lys-Leu-Gly-Ala-Pro-Ser-Ile-Thr-Cys-Val-Arg-Arg-Ala-Phe | Antimicrobial |           |
| Soy and Wheat     | Aspergillus sojae              | Gly-Tyr; Ala-Phe; Val-Pro; Ala-Ile; Val-Gly | Antihypertensive | [12]      |
| Soybean (Tempeh)  | Rhizopus oligosporus           | Ala-Val; Gly-Leu; Gly-Phe | ACE-inhibition | [2]       |
|                   |                                | Lys-Pro; Met-Tyr               | Antioxidants       |           |
| Casein            | Bifidobacterium longum KACC 91563 | Val-Leu-Pro-Val-Gln | Antioxidant | [20]      |

During the fermentation process, compounds are formed such as peptides and free amino acids. Cow’s milk fermentation (yoghurt) can describe peptide by SDS-PAGE and RP-HPLC with the different results [24]. Fractionation of peptides in Yoghurt by SDS-PAGE in figure 2.

![Figure 2. SDS-PAGE Yoghurt and Crude (Farvin, 2010).](image)

From the SDS-PAGE pattern in figure 2, This can be seen from baseband from protein material. The high molecular weight fraction > 30 kDa with BSA and Casein and < 30 kDa with β-lactoglobulin and α-lactobulmin part of protein in Milk Fermentation (yoghurt). Disadvantages of SDS-PAGE not clearly detect name fraction of protein. This causes camouflage in protein detecting and naming.
Figure 3. RP-HPLC profile of yoghurt. [24]

The Peptide analysis method is including principles and advantage in table 2.

| Method   | Principles                                                                                                                                                                                                 | Advantages/Disadvantages                                      | Reference |
|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|-----------|
| SDS-PAGE | peptide migration depends both on their molecular mass and on their net charge at the electrophoresis pH                                                                                            | A: Protein migrates until the decreasing pore size; Protein close with molecular weight values D: no clearly band protein | [21]      |
| RP-HPLC  | separates polypeptides based on subtle differences in the “hydrophobic foot” of the polypeptide being separated                                                                                         | Isolation of complex peptide mixture and clearly peptides Sensitivity separate peptides | [23]      |
| ESI/MS   | Based on the transformation of an aqueous solution with uniform electrical density to gas-phase ions, by passing a high voltage through a thin capillary.                                                      | Accurate measurement of the molecular weight of the peptides  | [25]      |

Fractionation of peptides in Yoghurt by RP-HPLC in figure 3. From the RP-HPLC peak, it is clear fractionation of the protein material in yoghurt. The TFA soluble fraction of the > 30 kDa fraction contained one major peak with long retention time (peak no. 32 at 41 min) confirming a high molecular weight. In the 10–30 kDa fraction, peaks with retention times between 25 and 40 min dominated. In the
fraction 3–10 kDa, the late eluting peaks (peaks no. 29–32) had been removed, and peaks with shorter retention times (10–25 min) were dominating [24]. RP-HPLC result confirmed the clear database than SDS–PAGE analysis that it can findings more lower molecular weight peptides in each fraction.

4. Conclusion
Fermented foods are biologically processed with microorganism produce bioactive peptides with a proteolytic enzyme. Bioactive peptide fermented food have health benefits such as antihypertensive, antioxidant and antimicrobial. Different peptides caused by different fermentation with bacteria or fungi and fermentation processes. Important techniques for identification peptide with different principles, mechanism, and advantage by SDS-PAGE, RP-HPLC and ESI/MS.

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