

The potential of buffalo (*Bubalus bubalis*) meat as a source of bioactive peptides using an *in silico* approach

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ABSTRACT

Buffalo meat (*Bubalus bubalis*) has great potential as a source of bioactive peptides, which play important physiological roles, including antihypertensive, antioxidant, and antidiabetic activities. This study aimed to apply an *in silico* approach as an efficient predictive strategy for assessing the bioactive peptide potential of buffalo meat, offering a practical alternative. Protein sequences were retrieved from UniProt, then analyzed using BIOPEP database to identify bioactive fragments. PeptideRanker was employed to predict the probability scores of bioactivity. Proteolytic activity was predicted using digestive enzymes (pepsin, trypsin, and pancreatic elastase) as well as additional enzymes (papain, proteinase-K, and thermolysin). The simulation results demonstrated that enzymatic hydrolysis generated peptides with various physiological activities. Hydrolysis with digestive enzymes produced the highest angiotensin-converting enzyme (ACE) inhibitory activity among the hydrolyzed samples. At the same time, papain and thermolysin also yielded fragments with potential as renin inhibitors. Proteinase-K exhibited relatively high activity, and several peptides showed the highest bioactivity scores, including those of an ACE inhibitor, antioxidant, renin inhibitor, and dipeptidyl peptidase IV (DPP IV) inhibitor. These findings indicate that buffalo meat is a promising candidate as a source of bioactive peptides with diverse physiological activities, supporting its potential utilization in the development of functional foods.

INTRODUCTION

Buffalo meat (*Bubalus bubalis*) has gained increasing popularity in Southeast Asia, the Middle East, Asia, and Africa due to its low fat and cholesterol content, absence of religious restrictions, and desirable quality attributes (El Debaky et al., 2019). It is one of the animal protein sources with significant potential to be developed as a raw material for bioactive peptides. Bioactive peptides are specific protein fragments that exhibit biological activities, such as antioxidant, antimicrobial, antihypertensive, and immunomodulatory properties (Ohata et al., 2016). Study also showed that they demonstrated strong potential to modulate gut microbiota and support gastrointestinal and overall health (Wijesekara et al., 2024). Research on bioactive

peptides has grown rapidly, particularly in the utilization of abundant animal protein sources in various regions, including buffalo, which is widely raised in tropical areas such as Indonesia. Buffalo meat possesses unique protein characteristics, with a favorable essential amino acid composition and relatively lower fat content compared to beef (Naveena et al., 2014). These attributes make buffalo meat a promising candidate for the development of bioactive peptides. Protein hydrolysis using specific enzymes, such as trypsin, pepsin, or papain, can generate bioactive peptides with diverse functional properties, which are relevant for applications in the functional food and pharmaceutical industries (Hartmann et al., 2007).

However, research on the exploration and characterization of bioactive peptides derived



from buffalo meat remains limited compared to other protein sources, such as milk, fish, or beef. Therefore, it is important to identify bioactive peptides from buffalo meat and evaluate their biological activities, including antioxidant and antihypertensive properties, which contribute to the development of buffalo meat-based functional food products.

An *in silico* approach was selected in this study due to its efficiency in the exploration and analysis of bioactive peptides from protein sources. This method enables the prediction of bioactive properties and peptide affinity toward biological targets without the need for labor-intensive and costly laboratory experiments. By utilizing bioinformatics tools and protein databases, the *in silico* approach can accelerate the identification of potential peptides exhibiting biological activities such as antioxidant or antihypertensive effects (Minkiewicz et al., 2008).

In addition, the *in silico* approach allows for the simulation of molecular interactions between bioactive peptides and biological targets, such as receptors or enzymes, with high accuracy. This technology not only provides quantitative predictions of peptide-target affinity but also offers detailed visualization of the molecular interaction mechanisms underlying their biological activities (Trott et al., 2010). Therefore, this approach is highly relevant for preliminary exploration studies, particularly for underexplored protein sources such as buffalo meat.

The advantages of the *in silico* approach also lie in its flexibility in handling protein data that is already available in public databases. There are some bioinformatics tools, such as ExPASy PeptideCutter, BIOPEP-UWM, and AutoDock Vina, utilized for *in silico* analysis (Duvaud et al., 2021; Trott et al., 2010). ExPASy or Expert Protein Analysis System, developed by the Swiss Institute of Bioinformatics, is a web-based platform providing integrated access to major protein and proteomics databases (Gasteiger et al., 2003). The BIOPEP-UWM™ database is a continuously updated and widely used tool for bioactive peptide research, offering expanding functionalities such as enhanced peptide annotation, quantitative bioactivity parameters, and identification of proteinases that release specific peptides (Minkiewicz et al., 2019). By utilizing those tools, this study can identify potential peptide fragments with high precision. The outcomes of

these analyses provide a strong foundation for subsequent *in vitro* or *in vivo* studies, which may ultimately support the development of buffalo meat-based functional food or pharmaceutical products.

Moreover, the *in silico* approach facilitates the simulation of molecular interactions between bioactive peptides and biological targets, such as receptors or enzymes, with high accuracy. This method enables the prediction of bioactive properties and peptide affinity toward biological targets without the need for time-consuming and costly laboratory experiments (Udenigwe et al., 2012). By leveraging bioinformatics software and protein databases, this approach can accelerate the identification of potential peptides exhibiting biological activities such as antioxidant and antihypertensive effects (Minkiewicz et al., 2008).

This technology not only provides quantitative predictions of peptide-target binding affinity but also offers detailed visualization of the molecular interaction mechanisms underlying their biological activities (Trott et al., 2010). Consequently, this approach is highly relevant for preliminary exploratory research, particularly for underexplored protein sources such as buffalo meat.

This study aims to isolate, identify, and characterize bioactive peptides generated from the enzymatic hydrolysis of buffalo meat proteins. An *in silico* approach was selected due to its efficiency in the exploration and analysis of bioactive peptides derived from protein sources.

MATERIALS AND METHODS

Collection of Buffalo Meat Protein Data

Protein sequence data from buffalo meat (*Bubalus bubalis*) were identified and retrieved from the UniProt protein database. The protein sequence with the accession code P84997 (MYG_BUBBU) was selected and evaluated for its potential biological activities. Buffalo meat (*Bubalus bubalis* L.) is characterized by a darker color compared to beef, which is primarily attributed to the concentration of myoglobin (Mb) and its oxidative state. Therefore, this sequence was chosen as it represents myoglobin, a key protein present in muscle tissue and closely associated with meat color and oxidative properties.

Bioactive Peptide Potential

The potential bioactive peptides derived from the selected protein sequence were analyzed using the BIOPEP database to identify their predicted bioactivities (Minkiewicz et al., 2008). The result of peptide sequences were subsequently ranked use the PeptideRanker software to evaluate their likelihood of exhibiting biological activity. PeptideRanker was used to predict the probability of amino acid sequences forming bioactive peptides. This tool assigns a score range from 0 to 1 for each peptide, where a higher score (closer to 1) indicates a higher likelihood of bioactivity, while a lower score (closer to 0) suggests minimal or no bioactive potential.

Enzymatic Hydrolysis

In silico proteolytic analysis was performed to simulate enzymatic digestion of the protein sequence used several digestive enzymes, include pepsin (pH 1.3; EC 3.4.23.1), trypsin (EC 3.4.21.4), and pancreatic elastase (EC 3.4.21.36), to mimic gastrointestinal digestion. In addition, non-digestive proteolytic enzymes, namely proteinase K, papain, and thermolysin, were also employed to explore alternative peptide generation patterns. The BIOPEP database was utilized to predict protein cleavage sites for each enzyme and to identify the potential bioactive peptides generated from the enzymatic hydrolysis (Minkiewicz et al., 2008).

RESULTS AND DISCUSSION

In silico methods involve the use of bioinformatics software and databases to analyze amino acid sequences. Through this approach, bioactive peptides can be predicted based on specific motifs or physicochemical properties associated with particular biological activities, such as antihypertensive, antioxidant, antimicrobial, or immunomodulatory effects.

Bioactive Peptide Potential

Based on the *in silico* analysis of the bioactive peptide potential of buffalo meat, several predicted activities were identified (Table 1), including ACE-inhibitory peptides, antioxidant peptides, dipeptidyl peptidase IV (DPP IV) inhibitors, dipeptidyl peptidase III (DPP III) inhibitors, and renin inhibitors.

ACE-inhibitory peptides function by

suppressing the activity of angiotensin-converting enzyme (ACE), which plays a key role in blood pressure regulation. Inhibition of ACE prevents the formation of angiotensin II, a potent vasoconstrictor, leading to vascular relaxation, reduced blood pressure, and a decreased risk

Table 1. Bioactive peptide potential of buffalo meat

Potency	Sequence	Value
ACE inhibitor	LGF	0.964
Antioxidative peptide	WG	0.993
DPPH IV inhibitor	GF	0.994
	WG	0.992
	FR	0.985
	AW	0.967
	WQ	0.909
DPP III inhibitor	GF	0.994
	FR	0.985
Renin inhibitor	WG	0.990
	LGF	0.960
	KF	0.906

Note: LGF= Leucine-Glycine-Phenylalanine; WG= Tryptophan-Glycine; GF= Glycine-Phenylalanine; FR= Phenylalanine-Arginine; AWA= Alanine-Tryptophan; WQ= Tryptophan-Glutamine; KF= Lysine-Phenylalanine.

of hypertension and cardiovascular diseases. In addition, peptides derived from buffalo meat also exhibit potential as dipeptidyl peptidase IV (DPP IV) inhibitors. DPP IV is involved in the degradation of incretin hormones such as glucagon-like peptide-1 (GLP-1). Inhibition of DPP IV prolongs the activity of GLP-1, thereby enhancing insulin secretion and reducing blood glucose levels, which is particularly beneficial in the management of type 2 diabetes mellitus. DPP III inhibitors suppress the activity of dipeptidyl peptidase III, an enzyme involved in the degradation of specific peptides and proteins. Although inhibition of DPP III may influence the biological activity of various bioactive molecules, the precise physiological roles of DPP III inhibitors remain to be further elucidated.

The second most prominent predicted activity was antioxidant activity. Antioxidant peptides are characterized by their ability to scavenge free radicals or prevent oxidative damage in cells and tissues. This activity is crucial for protecting the body against oxidative

stress associated with aging, inflammation, and various chronic diseases, including cancer and cardiovascular disorders. A literature review by Al Awwaly et al. (2015) reported that bioactive components in meat, such as bioactive peptides, can be generated through aging, fermentation, and enzymatic treatments. These peptides exhibit diverse functional properties, including antioxidants, antihypertensive, antimicrobial, anticancer, and immunomodulatory activities.

Furthermore, renin-inhibitory peptides act by inhibiting renin, a key enzyme in the renin-angiotensin system (RAS). By suppressing renin activity, these peptides prevent the formation of angiotensin I and angiotensin II, thereby contributing to blood pressure regulation and reducing the risk of cardiovascular diseases.

Enzymatic Hydrolysis

The percentage of degree of hydrolysis (DH) is a parameter that indicates the extent to which protein molecules have been hydrolyzed into their constituent units, such as peptides and amino acids. DH is commonly calculated based on the amount of soluble nitrogen in protein analysis or through measurable chemical changes, including pH variation or the concentration of free compounds (e.g., free amino acids). In the context of *in silico* analysis using software such as BIOPEP database, the degree of hydrolysis represents a theoretical simulation of protein cleavage by specific enzymes. BIOPEP database utilizes protein and enzyme databases to predict the extent to which proteins can be hydrolyzed into peptides or amino acids based on enzyme-specific

cleavage sites within the protein sequence.

In this study, buffalo meat proteins were hydrolyzed using pepsin to enhance angiotensin-converting enzyme (ACE) inhibitory activity, which plays an essential role in blood pressure regulation. One study investigating the bioactive peptide potential of buffalo meat was conducted by Sujarwanta et al. (2018). The results demonstrated that the purified hydrolysate of buffalo meat protein exhibited ACE-inhibitory activity with an IC_{50} value of $75.8 \pm 20 \mu\text{g mL}^{-1}$, indicating its potential as an antihypertensive agent.

Based on the analytical data above, digestive enzymes exhibited the highest degree of hydrolysis (%DH = 66.01) compared to the other enzymes, indicating high efficiency in breaking down proteins into peptides. The %DH value reflects the efficiency of an enzyme in cleaving protein substrates into peptide fragments. Digestive enzymes showed the highest %DH, suggesting that they are naturally optimized to degrade complex proteins. In contrast, thermolysin exhibited a lower % DH due to its narrower substrate specificity. This observation is consistent with the findings of Kitts et al. (2003) reported that naturally occurring proteolytic enzymes tend to be more efficient in generating peptide fragments than certain recombinant enzymes.

The bioactivity index (BE) provides an indication of the proportion of bioactive peptides generated during enzymatic hydrolysis. Proteinase-K exhibited the highest BE value for

Table 2. Values of enzymatic hydrolysis parameters

Enzyme	%DH	AE	W	BE	V	Activity
Digestive enzymes	66.01	0.1104	0.2267	5.674×10^{-4}	0.0664	ACE inhibitor
		0.0974	0.1485	9.272×10^{-5}	0.1394	dipeptidyl peptidase IV inhibitor
Papain	38.23	0.0519	0.1066	1.335×10^{-4}	0.0156	ACE inhibitor
		0.0130	0.2857	7.058×10^{-4}	0.9951	Renin inhibitor
		0.0844	0.1287	1.902×10^{-4}	0.2859	Dipeptidyl peptidase IV inhibitor
Thermolysin	31.84	0.0649	0.1333	5.982×10^{-4}	0.0700	ACE inhibitor
		0.0065	0.1429	7.058×10^{-4}	0.9951	Renin inhibitor
Proteinase-K	36.30	0.0455	0.0934	2.185×10^{-3}	0.2558	ACE inhibitor

Note: AE = level of enzymatic activity toward the protein substrate; W = number of peptides generated after simulated enzymatic protein hydrolysis; BE = bioactivity potential of the peptides produced after hydrolysis; V = number of enzyme-accessible cleavage sites within the protein

ACE-inhibitory peptides, highlighting its strong potential for antihypertensive applications (Korhonen et al., 2006). Thermolysin and papain showed high BE values for renin-inhibitory peptides, supporting their potential role in blood pressure reduction. High V values observed for papain and thermolysin in relation to renin inhibitors indicate that the target protein contains numerous relevant cleavage sites capable of generating these bioactive peptides. Conversely, the low V values for ACE-inhibitory peptides across most enzymes suggest that only a limited number of cleavage sites are suitable for producing ACE-inhibitory sequences. These findings are consistent with the study by Nongonierma et al. (2015) which emphasized that bioactive peptide generation is highly dependent on enzyme specificity toward the protein substrate.

A similar study by Minkiewicz et al. (2011) have employed the same *in silico* parameters to evaluate bovine meat proteins, demonstrating that these metrics consistently identify collagen and elastin as the richest sources of bioactive peptides, particularly ACE inhibitors, due to their high glycine and proline content and the superior peptide-releasing ability of broad-specificity enzymes such as proteinase K.

The results of this *in silico* analysis can serve as a valuable reference for designing subsequent laboratory-scale studies, as they provide preliminary insights and predictions regarding the outcomes of buffalo meat hydrolysis using selected enzymes. This approach has the potential to reduce the use of chemicals and other laboratory resources, as well as to save research time by offering prior predictions of enzymatic behavior. Although *in silico* analyses are not expected to be 100% accurate, their outcomes are often closely aligned with experimental results and can therefore be used as a reliable and practical guide.

However, several limitations should be acknowledged. The degree of hydrolysis (DH) values generated by BIOPEP database represent theoretical predictions based on enzyme characteristics and protein sequence data. Moreover, this approach does not account for environmental factors such as pH, temperature, or enzyme concentration, all of which significantly influence hydrolysis reactions under *in vitro* or *in vivo* conditions. Consequently, experimental validation remains essential to confirm the actual

degree of hydrolysis and the bioactive potential of the resulting peptides.

CONCLUSIONS

Based on *in silico* analysis, buffalo meat demonstrates a high potential as a source of bioactive peptides with predicted ACE-inhibitory enzyme, antioxidant, dipeptidyl peptidase IV (DPP IV) inhibitory, and renin-inhibitory activities. Enzymatic hydrolysis using digestive enzymes, namely the combination of pepsin, trypsin, and pancreatic elastase, resulted in the highest degree of hydrolysis and yielded the most prominent bioactive potential, particularly in the form of ACE-inhibitory peptides. These findings highlight the promising role of buffalo meat proteins as functional ingredients for the development of antihypertensive and health-promoting food products, while also providing a scientific basis for further *in vitro* and *in vivo* validation studies.

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