Moyo *et al.*
Food Production, Processing and Nutrition (2024) 6:90
https://doi.org/10.1186/s43014-024-00265-1

Food Production, Processing and Nutrition

REVIEW

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Urging bioactive peptide exploration in African fermented legumes: insights from microbial proteolysis to gastrointestinal stability

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Abstract

Microorganisms such as *Bacillus* spp., *Propionibacterium* spp., *Lactobacillus* spp., *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp, and *Aspergillus* spp. play vital roles in fermenting macromolecules present in African legumes, resulting in beneficial derivatives with diverse bioactivities advantageous to human health. While fermentation of legumes is a common practice in Africa, yielding nutritious products rich in phenolic compounds, the specific contribution of peptides generated during this process to the health-promoting qualities of legumes remains underexplored. This review aims to demonstrate the possibility of the occurrence of bioactive peptides in fermented African legume products by investigating the intricate processes underlying microbial conversion of proteins into peptides and explaining the structure–activity relationship governing their bioactivity. The review also evaluates the stability of bioactive peptides during digestion in the human gastrointestinal tract, shedding light on their potential health benefits. African fermented legume products could be utilized in various food systems such as condiments, meat binders and components of high-protein snacks, as sources of bioactive compounds in the production of functional foods and nutraceuticals. In summary, this comprehensive examination not only summarizes our understanding of the health-promoting qualities of fermented African legume products but also underscores their potential as sustainable food sources for commercial utilization in diverse food industries.

Keywords African fermented legumes, Bioactive peptides, Fermentation, Proteolytic activity, Bioactivity, Gastrointestinal tract stability

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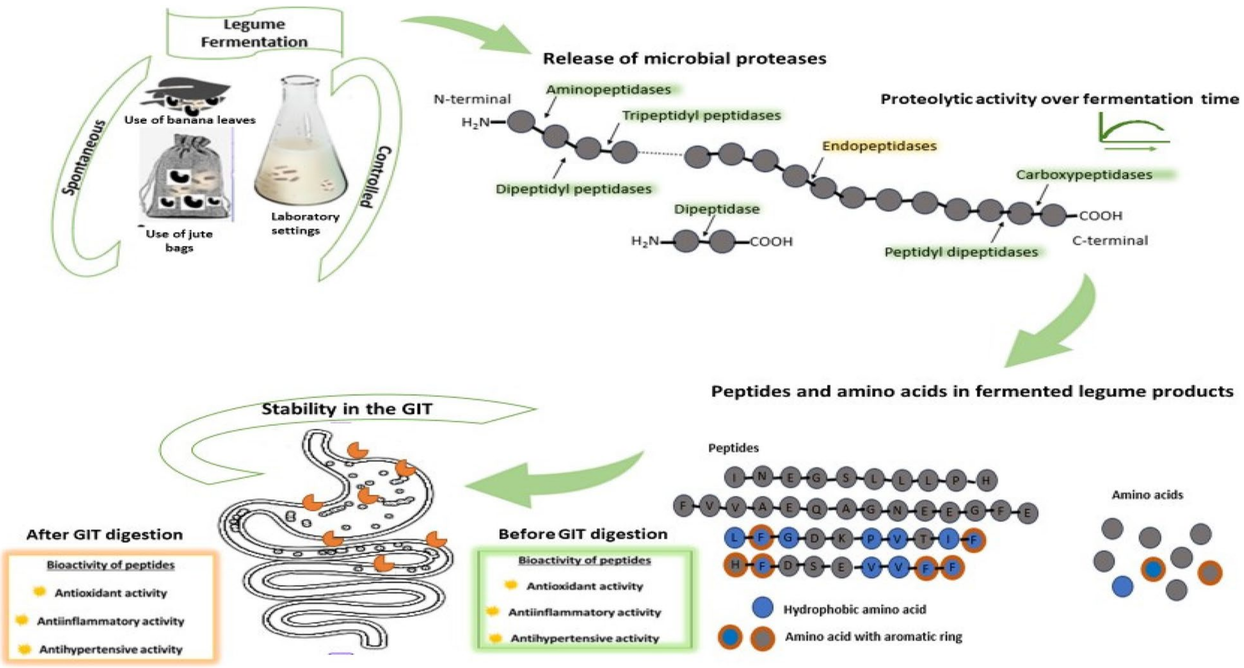
Graphical Abstract



Introduction

Fermentation is a food processing method proven to enhance nutritional content, extend shelf life, decrease cooking time, reduce flatulence-causing carbohydrates such as raffinose family oligosaccharides (RFOs), improve protein digestibility and diminish levels of antinutrients and allergenic compounds in legumes (Christensen et al., 2022; Seo & Cho, 2016; Materia et al., 2021; Parkouda et al., 2009). For example, trypsin inhibitors, hydrogen cyanide, saponin and oxalate were significantly ($p < 0.05$) decreased after 72 h *Rhizopus oligosporus* fermentation of the *Mucuna pruriens* legume (Ezegbe et al., 2023). A fermentation process utilizing legumes provides a sustainable approach to food production and environmental conservation, which can contribute towards socio-economic development (Maclean et al., 2011).

Legumes represent valuable sources of bioactive substances pivotal for enhancing human health in a sustainable dietary framework (Ibsen et al., 2022; Singh et al., 2021). Considering the escalating incidence of noncommunicable diseases (NCDs) such as cancers, cardiovascular diseases, and type 2 diabetes, with 41 million deaths reported globally each year (World Health Organization (WHO), 2022), there is a growing interest in harnessing bioactive peptides from sources such as legumes. These peptides offer promising avenues for combating oxidative stress, a key contributor to NCDs, as poor dietary practices exacerbate the risk of these diseases (World Health Organization (WHO), 2022). A redox imbalance can cause oxidative stress and inflammation, which could damage vital biomolecules within the body (Seyedsadjadi & Grant, 2021), and this necessitates the intake of dietary antioxidants such as bioactive peptides (Paula et al., 2022; Wei et al., 2023).

Bioactive peptides, characterized by molecular weights below 10 kDa, are encoded within various dietary proteins that exert their biological activities on release from their parent proteins (Mora et al., 2019). These peptides may be derived through the action of microbial extracellular proteases, which are enzymes either bound to the microbial cell envelope or secreted into the fermentation medium (Christensen et al., 2022). The proteases encompass both exopeptidases and endopeptidases, and cleave peptide bonds at either the ends or within the internal sections of polypeptide chains, which generates a diverse array of peptides in the fermentation environment (Raveendran et al., 2018). For example, certain strains of *Bacillus* spp. are known to secrete alkaline proteases, including serine proteases and neutral metalloproteases (Mantsala & Zalkin, 1980), yeasts produce aminopeptidases and carboxypeptidases (Mirzaei et al., 2021), and lactic acid bacteria (LAB) release serine protease, zinc metalloprotease and aminopeptidase C (Christensen et al., 2022; Juillard et al., 2021).

There is a reasonable case for the study of the role of peptides generated by microbial enzyme proteolytic activity during fermentation of African legumes, as there is a paucity of data regarding their health-conferring benefits. The bioactivities commonly reported are frequently ascribed to the presence of phenolic compounds. Studies have linked the antioxidant activities of cowpea (*Vigna unguiculata*), African yam bean (*Sphenostylis stenocarpa* Harms) and bambara groundnut (*Vigna subterranea* L. Verdc) to the phenolics present in fermented samples (Kapravelou et al., 2014; Oboh et al., 2008, 2009). Despite these findings, recent studies have proved the role of peptides in antioxidant activity, antihypertensive activity, anti-obesity and anticancer activity found in non-African fermented legumes such as the common bean, soybean, bitter bean, pea and red bean (Jakubczyk et al., 2017; Muhialdin et al., 2020; Vermeirssen et al., 2003; Wei et al., 2023; Xiao et al., 2018). The efficacy of bioactive peptides derived from fermented legumes centers on their stability, bioaccessibility in the gastrointestinal tract (GIT) and subsequent bioavailability, as these peptides need to be resistant to the protease digestive enzymes in the GIT for their bioactivity to be retained (Pei et al., 2022).

This review proposes to highlight the necessity for research into the potential bioactive role of peptides present in African fermented legumes. It will explore the proteolytic activities responsible for releasing various bioactive peptides and investigate their structure–activity relationship. Additionally, the review will evaluate the stability of bioactive peptides derived from fermented legumes in the gastrointestinal tract. By emphasizing the significance of bioactive peptides in fermented legumes and their stability, the review aims to advocate for their utilization in commercial food processes, such as in the production of condiments, meat binders and high-protein snacks. It also advocates their use as sources of bioactive compounds in the production of functional foods and nutraceuticals, which can promote public health improvements.

Overview of fermented legumes in Africa, fermentation methods and inherent microorganisms

Various legumes (cultivated exotic species or species indigenous to Africa) have historically been subjected to either spontaneous/traditional, controlled, or back-slopping fermentation techniques in Africa (Fig. 1). Spontaneous fermentation is a commonly used traditional method. Both solid-state substrates and submerged fermentation are used. The dominant microorganisms and fermentation conditions then determine the trajectory of fermentation to an alkaline or acidic pH. As shown in Table 1, most spontaneously fermented legumes are characterized by alkaline fermentation in which *Bacillus* spp. dominate. Some studies have used mixed inocula consisting of *Lactobacillus* spp., *Bacillus* spp., filamentous fungi spp. and *Phycomycetes* spp. for controlled fermentation.

The processing technique employed prior to spontaneous fermentation often involves soaking the legume seeds for 12–24 h in water to enable the subsequent dehulling process. Dehulling is a necessary step as legumes are known to contain antinutrients (phytates, lectins and protease inhibitors) in their seed coat which could inhibit ideal fermentation conditions. The seeds are then boiled for 15 min to 2 h or more depending on the toughness of the bean cotyledon. During boiling the plant cell walls are degraded, which facilitates access to the microbial enzymes. In addition, the process gelatinizes starch which allows microorganisms to efficiently utilize the starch as a source of energy and carbon. The boiling also assists in eradicating harmful microorganisms and antinutrients. Boiling may also activate the germination of beneficial *Bacillus* spp. Spores which can enhance the overall fermentation process (Luu et al., 2015). After boiling, the legumes are drained, typically wrapped in clean clothes, jute bags or banana leaves (*Musa sapientum* Linn.) (Okwunodulu & Agha, 2020), and left to ferment at room temperature for several days (2–21). The wrapping material assists in controlling humidity and gradually increasing the temperature of the fermenting legumes. This practice also selectively influences the type of microorganisms which will grow as the fermentation proceeds. Various microorganisms are present at different stages of spontaneous fermentation (Senanayake et al., 2023).

It must be emphasized that during fermentation processes, it is crucial to maintain hygienic conditions and employ appropriate sanitation practices. Certain spontaneously fermented products (Table 1) may harbour pathogenic microorganisms such as *Staphylococcus aureus* and *Bacillus cereus*, which are known to cause foodborne illnesses (Agunwah et al., 2024; Chin et al., 2024). Environmental factors such as temperature, pH and relative humidity may permit the colonization of fungi such as *Aspergillus parasiticus* and *Aspergillus flavus*, which can generate Aflatoxin B1, a toxic substance harmful to humans (Humza et al., 2021; Olagunju & Ijabadeniyi, 2021). It is therefore important to develop safe fermentation practices, with stable starter cultures and controlled fermentation that enables the regulated addition of selected microorganisms.

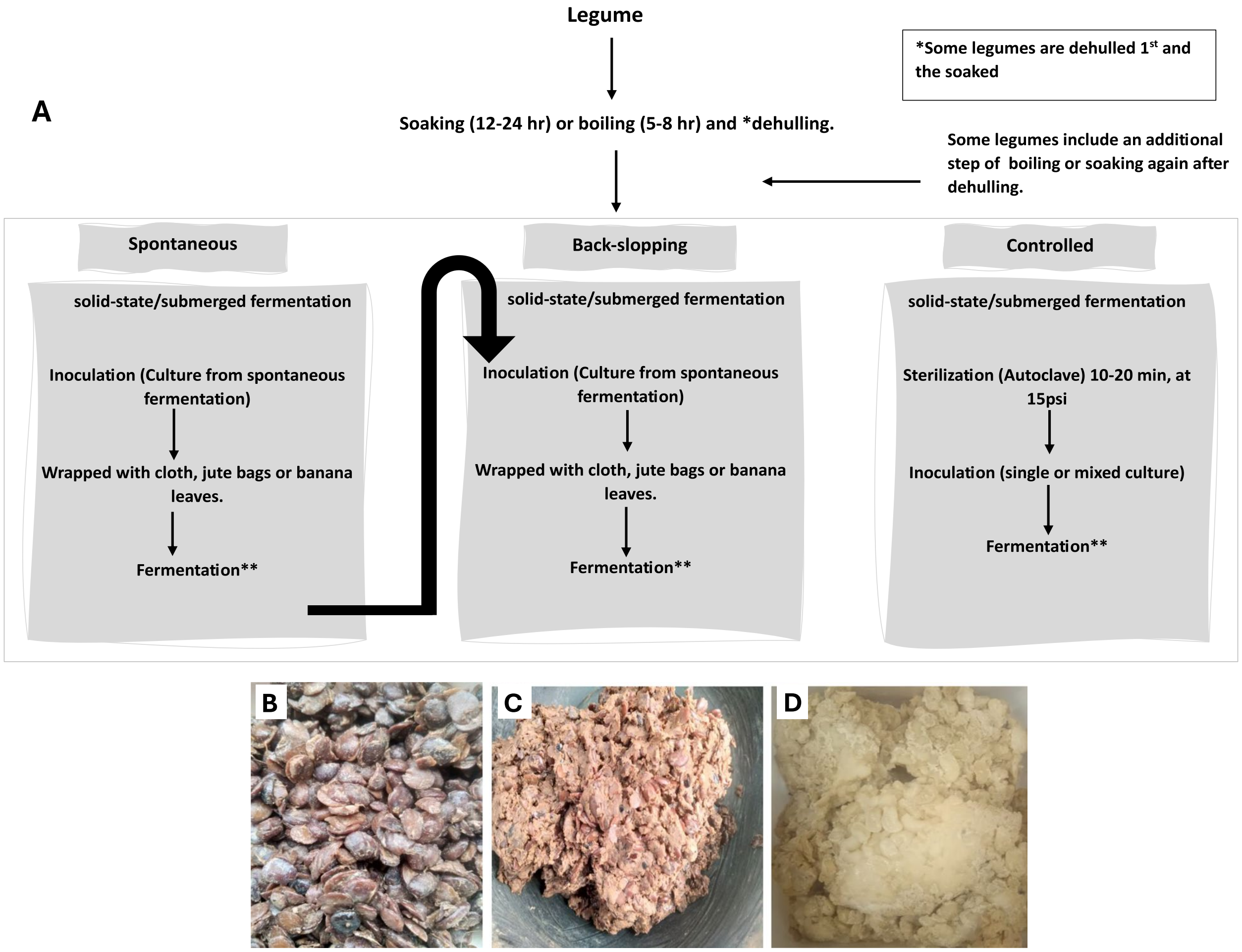


Fig. 1 **A** Shows processing techniques used in African legume fermentation. **B**, **C** Show spontaneously alkaline fermented African locust bean (*Parkia biglobosa*). **D** Shows spontaneously alkaline fermented cowpea (*Vigna unguiculata*)

In controlled fermentation, seeds submerged in water or slurry are autoclaved at 121 °C for 10–20 min, and an inoculum of 10^6–10^8 cells/mL at a range of 2%-10% (v/v) is added (Azeke et al., 2005; Dueñas et al., 2005; Isu & Ofuya 2000; Kapravelou et al., 2014; Limón et al., 2015). The autoclaving process is conducted to eradicate any microbes present in the substrate, but can cause Maillard reactions and affect the growth of inoculated microorganisms. In this event the fermentation medium temperature (26 °C to 45 °C) is then usually set according to the type of microbe inoculated into the substrate, as presented in Table 1.

Alkaline-fermented beans are characterized by a strong ammonia odour and sticky grayish mucilage, which is a mixture of poly gamma glutamic acid (PGA) and fructan. Acid-fermented products are characterized by a sour taste, among other attributes depending on the type of microorganism present. Based on country and legume type, these fermented legume products are identified by different names, such as Ugba (Nigeria), Iru (Nigeria and West Africa), Dawadawa (Ghana and Nigeria), Siljo (Ghana, Nigeria and Senegal), Soumbala (Burkina Faso, Mali and Ghana), Netetu (Senegal and Gambia), Aitin (West Africa) Kinda (Ghana and Nigeria), Owoh (Nigeria), Okpehe (Nigeria), Kpaye (Benin and Togo) and Soydaddawa (Nigeria and other West African countries).

Important characteristics of parent proteins in legumes commonly used for fermentation in Africa

The microbial utilization of proteins as a substrate may be influenced by seed protein structural characteristics. These characteristics vary within legumes, and differences may affect the physiochemical properties of the proteins and their subsequent utilization in food systems. Legume proteins can be classified according to gene ontology (GO) into three domains, biological process, cellular component and molecular function

Table 1 Fermented legumes in Africa and microorganisms identified

Legume	Fermentation type and method	Fermentation conditions			Microbial species present	References
		Temperature (°C)	Time	pH (initial -final)		
African oil bean (*Pentaclethra macrophylla Bentham*)	Spontaneous	Room	72 h	-	*Lactobacillus* spp., *Bacillus* spp., *Pseudomonas* spp *and Staphylococcus* spp.	(Eluchie et al., 2021)
	Controlled	35	72 h		*Bacillus subtilis* and *Bacillus megaterium*	
	Spontaneous	-	5 days	-	*Staphylococcus* spp., *Escherichia coli, Bacillus* spp., *Micrococcus* spp., *Pseudomonas* spp., *Proteus* spp., *Saccharomyces* spp., *and Candida* spp	(Itaman & Nwachukwu, 2021)
	Alkaline-Spontaneous	TF	TF	TF	*Bacillus subtilis*, *Bacillus coagulans*, *Bacillus pumilus* and *Bacillus megaterium*	(Isu & Ofuya, 2000)
	Alkaline-Controlled	30	6 days	5.9–6.9	*Bacillus subtilis*	
	Alkaline-Controlled	37	72 h	6.58–6.78	*Bacillus subtilis*	(Enujiugha et al., 2008)
				6.60–6.68	*Bacillus licheniformis*	
				6.60–6.62	*Pseudomonas fluorescens*	
				6.62–7.38	Mixed culture (*Bacillus subtilis*, *Bacillus licheniformis*, *Pseudomonas fluorescens*)	
African locust bean (*Parkia biglobosa*)	Alkaline-Spontaneous	TF	TF	TF	*Bacillus subtilis*, *Pseudomonas fluorescens, Bacillus licheniformis, and Micrococcusspecies*	(Oguntimehin et al., 2023)
		TF	TF	TF	*Bacillus licheniformis*, *Bacillus subtilis and Bacillus cereus*	(Olajuyigbe & Ajele, 2008)
		26–34	5 days	6.2–7.0	*Bacillus subtilis*, *Lactiplantibacillus plantarum, Corynebacterium diphtheria, Actinomyces* spp., *Bacillus pumilis, Lactobacillus lactis, Bacillus cereus, Rhyzopus stolonifer, Rhyzopus oryzae, Rhyzopus oligosporus and Aspergillus flavus*	(Osuntokun et al., 2020)
		25–33	72 h	5.44–6.35	*Bacillus subtilis*, *Bacillus polymyxa, Staphylococcus aureus, Leuconostoc mesenteroides, Rhizopus stolonifera, Mucor mucedo*	(Adegbehingbe & Daramola, 2019)
African locust bean (*Parkia biglobosa*) and Lima bean (*Phaseolus lunatus*)	Acidic-Spontaneous	26–31	72 h	5.74–5.20	*Bacillus subtilis*, *Bacillus polymyxa, Lactobacillus casei* *Staphylococcus aureus, Aspergillus flavus, Penicillium expansum*	(Adegbehingbe & Daramola, 2019)

Table 1 (continued)

Legume	Fermentation type and method	Fermentation conditions			Microbial species present	References
		Temperature (°C)	Time	pH (initial -final)		
African yam Bean (*Sphenostylis stenocarpa*)	Acidic-Mixed	30	48 h	-	*Lactiplantibacillus plantarum*	(Azeke et al., 2005)
	Alkaline-Spontaneous	33–46	72 h	6.8–7.52	*Bacillus pumilis, Bacillus licheniformis, Bacillus subtilis, Staphylococcus* spp.	(Ogbonna et al., 2001)
	Controlled	30	120 h		*Bacillus amyloliquefacien, Bacillus subtilis and Bacillus siamensis*	(Okolie et al., 2023)
Cowpeas (*Vigna unguiculata*)	Controlled		72 h		*Lactiplantibacillus plantarum, Oenococcus oeni, Saccharomyces cerevisiae, Acetobacter aceti*	(Ferreira et al., 2019)
Bambara groundnut (*Vigna subterranean*)	Alkaline-Spontaneous	30	4 days	7.0–8.0	*Bacillus subtilis, Bacillus licheniformis, Bacillus licheniformis*	(Amadi et al., 1999)
	Alkaline-Spontaneous	30	2 days		*Bacillus licheniformis*	
	Alkaline-Controlled	30, 37 and 45	4 days	~6.6 – 8.7		
Groundnut (*Arachis hypogaea* L.)[a]	Spontaneous		21 days		*Bacillus* spp., *Propionibacterium* spp., *Lactobacillus* spp., *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp.	(ElGasim & Abaker, 2012)
Jack bean (*Canavalia ensiformis*)	Controlled		7 days		*Limosilactobacillus fermentum* strain LF4801 *Pediococcus pentosaceus* strain PP4601 *Bacillus polymyxa* strain BP 1801, *Alcaligenes faecalis* strain AF1803, *Klebsiella ozaenae* strain KO1802 and *Rhizopus oryzae* strain MF4801, *Mucor mucedo* strain MF4802, *Aspergillus niger* strain MF4803, *Varicosporium elodea* strain MF4804, *Neurospora crassa* strain MF4806, *Brettanomyces bruxellensis* strain YF2401, *Schizosaccharomyces pombes* strain YF2404	(Gabriel-Ajobiewe, 2011)
Kersting's Groundnut (*Macrotyloma geocarpum* (Harms))	Spontaneous		3 days		*Bacillus licheniformis, Bacillus subtilis, Bacillus megaterium, Bacillus polymyxa, Bacillus coagulans, Lactobacillus plantarum, Staphylococccus aureus, Aspergillus niger, Rhodotorula* spp., *Aspergillus parasiticus, Rhizopus stolonifer and Saccharomyces cerevisiae*	(Abiola & Oyetayo, 2016)
Lima bean (*Phaseolus lunatus*)	Acidic-Spontaneous	26–31	72 h	5.78–4.63	*Bacillus subtilis, Bacillus polymyxa, Lactobacillus casei, Micrococcus rubens, Staphylococcus aureus, Aspergillus flavus, Penicillium expansum*	(Adegbehingbe & Daramola, 2019)

Table 1 (continued)

Legume	Fermentation type and method	Fermentation conditions			Microbial species present	References
		Temperature (°C)	Time	pH (initial -final)		
Mesquite *Prosopis africana*	Spontaneous		3 days		Not mentioned	(Odibo et al., 2008)
		TF	TF	TF	*Bacillus atrophaeus/velezensis*, *Bacillus tequilensis/cabrialesii*, *Bacillus cereus/paramycoides*, *Bacillus amyloliquefaciens/siamensis*, *Bacillus lichenformis/haynesii*, *Bacillus anthracis*, *Bacillus siamensis*, *Staphylococcus ureilyticus/cohnii*, *Staphylococcus nepalensis*, *Staphylococcus simulans*, *Escherichia fergusonni*, *Shigella flexneri*, *Oceanobacillus caeni*, *Paenibacillus barcinonensis*, *Lynsinibacillus xylanilyticus*, and *Vagacoccus lutrae* (Identified fermented Mesquite products from different markets)	(Agunwah et al., 2024)
Mung bean (*Vigna radiata* L.)	Spontaneous		7 days		*Bacillus* spp., *Staphylococcus aureus*, *Leuconostoc mesenteroides*, *Lactiplantibacillus plantarum*, *Saccharomyces cerevisae*, *Aspergillus* spp., *Penicillium italicum*	(John & Olusegun, 2016)
Pigeon pea (*Cajanus cajan*)^a	Spontaneous		96 h		*Bacillus subtills*, *Bacillus megatarium*, *Staphylococcus* Spp, *Saccharomyces cerevisae*	(Oyarekua, 2011)
Soybeans (*Glycine max* (L.) *Merr.*)	Alkaline Spontaneous	TF	TF	TF	*Bacillus tequilensis*, *Nosocomiicoccus ampullae*	(Ezeokoli et al., 2016b)
		29	72 h	7.06–8.19	*Bacillus subtilis*, *Bacillus safensis*, *Bacillus anthracis*, *Enterobacter hormaechei*, *Enterococcus casseliflavus*, *Staphylococcus sciuri*, *Staphylococcus xylosus*, *Bacillus tequilensis*, *Bacillus methylotrophicus*, *Bacillus siamensis*, *Enterobacter hormaechei*	(Ezeokoli et al., 2016a)
Tamarind (*Tamarindus indica* L.)	Spontaneous		72 h	-	*Bacillus subtilis*, *Bacillus pumilus*, *Bacillus licheniformis* and *Staphylococcus* spp	(Olagunju et al., 2018)
Velvet bean (*Mucuna pruriens*)	Controlled	29	72 h	-	*Rhizopus oligosporus*	(Ezegbe et al., 2023)

[a] Exotic but cultivated in Africa, *TF* Traditionally fermented product that was sourced from a Market

(Capriotti et al., 2014). Storage proteins (globulins, albumins, prolamins and glutelins) which belong to the molecular function domain, occur in large quantities (70 – 83%) in the seed and serve as nutritional reservoirs (Capriotti et al., 2014). Globulins, which are soluble in salt solutions, are the most abundant seed proteins (70–80%) and are categorized by their sedimentation coefficients (S) 2S, 7S, 11S, 12S and 15S, with 7S (vicilin) and 11S (legumins) constituting the major portion of globulins (Bennetau-Pelissero, 2019; Shevkani et al., 2019). The 2S proteins comprise globulins and albumins (Srivastava, 2002), although the coefficient S can vary based on extraction conditions and species (Srivastava, 2002).

In the mature seed, the 11S legumin is a hexamer composed of 6 subunits. Each subunit consists of two polypeptides (α acidic and β basic units) linked by a disulfide bridge (Srivastava, 2002). These legumins possess more sulphur-rich amino acids (methionine and cysteine) than vicilins (Neji et al., 2022). The 7S vicilin is a trimer of polypeptides (α, α’ and β) which can be identical or nonidentical and are linked by noncovalent hydrophobic interactions (Shevkani et al., 2019). These vicilins lack disulfide bonds due to the absence of cysteine in their structure, and are frequently glycosylated (Shevkani et al., 2019). In *Phaseolus* spp., such as in the lima bean, the predominant globulin is phaseolin (56.20%) (Palupi et al., 2022), in *Vigna unguiculata* spp. (cowpea), the predominant globulins are α-vignin, β-vignin and γ-vignin (López-Barrios et al., 2014), and the predominant proteins in *Glycine max* (soybean) are β-conglycinin (7S vicilin) and glycinin (11S legumin) (Aguirre et al., 2014).

Albumins, which are soluble in water, constitute 10–20% of legume seed proteins and consist of 4 kDa and 9 kDa polypeptide chains linked by disulfide bonds (Day, 2013; Shevkani et al., 2019). Albumins consist of proteins such as lectins, amylase inhibitors and protease inhibitors (Arntfield & Maskus, 2011) which possess higher amounts of cysteine and methionine than globulins. *Vigna subterranea* (bambara groundnut) proteins mostly consist of albumins (14–71%), while cowpea, lima bean and soybean contain 20–25%, 18.47% and 10–20%, respectively (Gulzar & Minnaar, 2017; Palupi et al., 2022). Overall, legumes are considered a poor source of sulphur-containing amino acids and tryptophan (Bennetau-Pelissero, 2019).

Protein secondary structure can be described in terms of the composition of α-helixes, β-sheets, β-turns, irregular coils and random coils (Yu et al., 2024). The secondary structure of legumes is mainly composed of β-sheets with few α-helices (Neji et al., 2022). A higher ratio of β-sheets to α-helixes can cause low protein digestibility, among other factors (Shevkani et al., 2019). For the tertiary structure, protein subunits can be arranged, connected and maintained by the presence of noncovalent bonds such as salt bridges, hydrogen bonds and disulfide bonds (Yu et al., 2024).

Microbial degradation of protein and release of peptides during legume fermentation

Several reactions occur during fermentation to modify complex substrates into simple compounds (Christensen et al., 2022) such as peptides, amino acids and ammonia (via proteolysis), fatty acids (via lipolysis) and monosaccharides (via degradation of poly and oligosaccharides) (Kouakou et al., 2021). For proteins to undergo degradation into peptides and amino acids, the peptide bonds must be made accessible for microbial proteolytic enzymes. Denaturation, which involves unfolding or altering the conformation of proteins, can be induced by changes in pH and temperature. These changes can disrupt hydrogen bonds and other noncovalent interactions crucial for maintaining intricate protein structures such as the complex quaternary structure (globulins), tertiary structures and secondary structures like β-sheets and α-helices. The use of an appropriate processing technique before fermentation of legumes may enhance the proteolytic ability of microbial enzymes during fermentation (Neji et al., 2022; Salazar-Villanea et al., 2016). When denaturation occurs, the peptide bonds are exposed to hydrolysis by proteolytic enzymes, during which the protein is hydrolyzed into macromolecular peptides, small molecule peptides and amino acids (Mu et al., 2024).

Microorganisms present in the substrate may produce extracellular proteolytic enzymes that could be bound to the microbial cell envelope, or released into the fermentation environment (Christensen et al., 2022) for the hydrolysis of proteins and subsequent release of peptides and amino acids, as illustrated in Fig. 2. In some strains the proteolytic system includes a cell envelope proteinase (CEP), which allows extracellular proteins to be broken down into peptides short enough to be taken up by peptide transport systems (oligopeptide permease (Opp), ion-linked transporter (DtpT), ABC transporter (Dpp)) (Christensen et al., 2022; Kayitesi et al., 2023). *Lactobacillus helveticus* LMG 11474 was found to release more of its proteases into the fermentation medium for the fermentation of pea protein, rather than utilizing cell-bound proteases (Vermeirssen et al., 2003). The *Bacillus subtilis* group may produce multiple extracellular proteinases (Li et al., 2023). Harwood & Kikuchi, (2022) found that the group encodes eight extracellular proteases, of which five are serine proteases and three are metalloproteases. Filamentous fungi, like the *Aspergillus oryzae* used in the

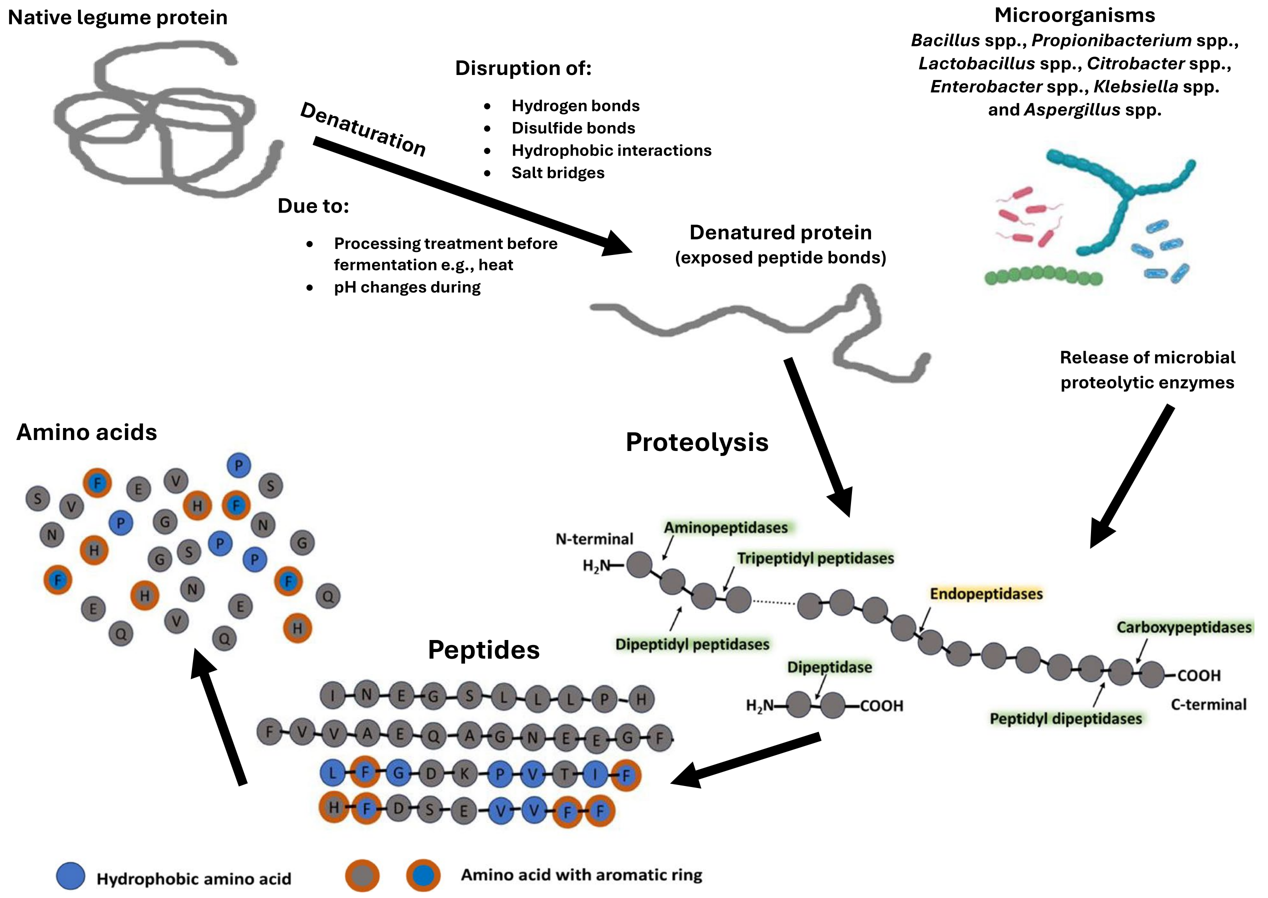


Fig. 2 Model illustrating microbial degradation of proteins during legume fermentation

production of soy sauce during solid-state fermentation, have produced 27 potential key proteolytic enzymes, organisms which are also known to release extracellular proteases (Mu et al., 2024).

All proteins present in legume seeds are potential sources of peptides (Garmidolova et al., 2022) although these can vary in length (2–20 amino acids) and molecular weight (≤ 10 kDa). Glycinin was found to generate approximately 95% of the peptides produced during soybean fermentation with *Bacillus subtilis* ATCC 41332 and *Rhizopus oligosporus* NRRL 2710 (Gibbs et al., 2004). In contrast *Lactobacillus helveticus* CRL 1062, *Lactobacillus delbrueckii* subsp. *lactis* CRL 581 and *Limosilactobacillus reuteri* CRL 1099 have been found to hydrolyze more β-conglycinin soybean protein for peptide production (Aguirre et al., 2014).

Numerous studies have shown the presence of peptides in fermented legumes, including red bean (Xiao et al., 2018), soybean (Wei et al., 2023), bitter bean (Muhialdin et al., 2020), pea (Vermeirssen et al., 2003) and common bean (Jakubczyk et al., 2017). Peptides such as EAKPSFYLK, AIGIFVKPDTAV, PTEMGLDVFQSRAN, PFGNNLLTVISGSAERAPTL, PVNNNAWAYATNFVPGK, PVANNAWAYATNFVPGQ and YLDAIGIFVKPDTAV have been identified in bitter beans after *Limosilactobacillus fermentum* ATCC9338 solid-state fermentation (Muhialdin et al., 2020). A 3 h fermentation of common bean by *Lactiplantibacillus plantarum* 299v at 22 °C was found to hydrolyze vicilin and legumin J-like proteins, resulting in the production of peptides such as INEGSLLLPH and FVVAEQAGNEEGFE, respectively (Jakubczyk et al., 2017). Despite these advances across the world, there is very little data regarding peptides produced via fermentation of the African oil bean, African locust bean, African yam bean, cowpeas, bambara groundnut, jack bean, kersting's groundnut and lima bean.

Some evidence of peptide presence after fermentation of certain of these legumes exists. Oyedoh et al. (2020) revealed a considerable concentration of peptides after cowpea and groundnut fermentation by *Lactiplantibacillus plantarum* CAU4347. The highest peptide concentrations of 80 µg/ml and 60 µg/ml, respectively, were obtained after 48 h of fermentation. The authors concluded that these peptide concentrations depended on the proteolytic activity of the microorganisms involved.

Protease release, classification and proteolytic activity of microorganisms during fermentation of legumes

Proteases released by microbes are classified based on the pH (neutral, acidic or alkaline) at which they are active (Bustamante-Torres et al., 2021; Razzaq et al., 2019). They can also be classified based on their functional groups or the points at which peptide bonds are cleaved (Razzaq et al., 2019). Exopeptidases (aminopeptidase, dipeptidyl peptidase, tripeptidyl peptidase, carboxypeptidase, peptidyl dipeptidase and dipeptidase) cleave the peptide bond close to the amino or carboxy termini of the polypeptide, while endopeptidases cleave internal bonds in polypeptide chains (Jisha et al., 2013), as illustrated in Fig. 2. The endopeptidases are further classified into six groups, based on the catalytic residue present in the active site, namely, serine, aspartic, cysteine, metallo, glutamic acid and threonine proteases (Raveendran et al., 2018). Endopeptidase generally releases macromolecular peptides rather than amino acids, while exopeptidase is mainly associated with the release of small molecule peptides such as dipeptide, tripeptide and amino acids (Mu et al., 2024).

The type of enzyme released by a microorganism will determine the cleavage of the proteins available in the legume substrate and the consequent types and sizes of peptides produced. Aminopeptidase N (PepN) is an exopeptidase with broad specificity, but it mostly tends to remove alanine and leucine residues from small peptides (Chandu & Nandi, 2003). Aminopeptidase A (PepA) is specific to the cleavage of N-terminal aspartic (Asp) and glutamic (Glu) acids, and to a lesser extent, serine residues (Ewert et al., 2017). Oligopeptidase (PepO) has a high affinity for larger peptides and a preference for peptide bonds with a hydrophobic amino acid in the P1 position (Kok, Mierau, & Monnet, 2013). A study by Verni et al. (2017) investigated the peptidase activities (PepN, PepA and PepO) of various LAB (*Enterococcus spp., Enterococcus casseliflavus, Lactococcus lactis, Lactobacillus sakei, Leuconostoc mesenteroides, Pediococcus* spp., *Pediococcus pentosaceus, Weissella cibaria and Weissella koreensis*) isolated from Finnish and Italian faba bean sourdoughs. *Pediococcus pentosaceus* F77 was found to possess a high PepN activity of 2.472 U, accompanied by a low activity of PepA and PepO, while *Enterococcus* spp. F09 had a higher PepA activity (1.650 U) and lower PepN and PepO activities.

The study of proteolytic activity of microorganisms can assist in optimizing fermentation conditions to produce bioactive peptides. Proteolytic activity during the fermentation of some African legumes has been studied, as indicated in Table 2. The proteolytic activity was either measured directly from the crude enzyme extract at specific times during the fermentation period or from microorganisms isolated during fermentation and cultured on different growth media. The presence of amino acids, proteins and carbohydrates may influence the expression of the proteolytic system of microbes present during fermentation (Venegas-Ortega et al., 2019), due to the regulation of gene expression in response to their environmental conditions (Kieliszek et al., 2021). The proteolytic activity of *Lactiplantibacillus plantarum* CAU4347 was found to be higher in fermented cowpeas than in fermented groundnuts (Oyedoh et al., 2020). The groundnut was reported to have higher protein contents than cowpea, although the process of autoclaving might have increased the amount of available nitrogen. The proteolytic system is regulated in response to changes in available nitrogen in the substrate to maintain the correct nitrogen balance (Kieliszek et al., 2021).

Proteolytic activities may be affected by the method of processing (boiling or roasting of legumes) before fermentation. For example, pre-boiled soy-dawadawa exhibited higher proteolytic activities during fermentation (2.36 Units (U)) compared to pre-roasted soy-dawadawa (1.96 U) (Opai-Tetteh, 1999). This could be attributed to the differences in moisture content and protein concentration of the boiled and roasted soy-dawadawa. The boiling and roasting processes could have impacted changes in the protein structure (intramolecular/intermolecular β-sheets and globular protein subunits). Research on the common bean (*Phaseolus vulgaris* L.) has noted more intermolecular β-sheet aggregates in moist heated samples than in dry heated samples (Choe et al., 2022). Intermolecular β-sheet aggregates are formed during heating and influence lower protein digestibility (Choe et al., 2022). The boiling process also may have increased the opening of the soy-dawadawa cell wall, thereby making the proteins much more accessible to the proteases than in the roasted soy-dawadawa. The moist-heating approach may cause portions of the cell wall's middle lamellae to separate as the pectin breaks down, increasing protein digestibility (Choe et al., 2022). Microbial growth stages may also influence their proteolytic activity. For example, *Bacillus licheniformis* LBBL-11 isolated from an African locust bean was found to have the highest proteolytic activity (18.4 Units/milliliter (U/ml)) at a point when bacterial growth was at its highest, i.e., after 48 h of growth (Olajuyigbe & Ajele, 2008). This could be because during the log phase microbial growth surges, which increases the demand for nutrients, thereby increasing proteolytic activity for the supply of nitrogenous compounds.

Table 2 Proteolytic activity identified during fermentation of legume products or from microorganisms present in legume fermentation

Product (Legume)	Processing conditions before fermentation	Fermentation time (h)	pH	Temperature (°C)	Proteolytic activity	Microorganisms identified/used	References
					U/ml		
(Cowpea)	Autoclaved	0	6.40	37	0	*Lactobacillus plantarum* CAU4347	(Oyedoh et al., 2020)
		24	5.91		~125		
		48	5.68		~20		
		72	5.36		~15		
(Ground nut)	Autoclaved	0	6.40		0	*Lactobacillus plantarum* CAU4347	
		24	5.65		~80		
		48	5.46		~10		
		72	5.19		~5		
					U/ml		
(Ground nut)	-	0	9	45	0	*Bacillus subtilis* SHS-04	(Olajuyigbe & Ajele, 2008)
		12	9		~125		
		24	9		~250		
		36	9		~310		
		48	9		~450		
		60	9		~360		
		72	9		~150		
		84	9		~80		
					mol/sec		
Dawadawa (African locust bean)	Boiled	0	-	ND	~0.00225	NI	(Evans et al., 2009)
		24	-		~0.00223		
		48	-		~0.00224		
		72	-		~0.0026		
		96	-		~0.0028		
		120	-		~0.0027		
					U/ml		
Iru (African Locust Bean)	Product obtained from the market	0		ND	0	*Bacillus licheniformis* Lbbl-11	(Olajuyigbe & Ajele, 2008)
		24			10		
		48			18		
		72			16		
					U		
Ogiri-okpei (Mesquite)	Boiled	0	-	Room temperature	0	NI	(Odibo et al., 2008)
		24	-		~0.5		
		48	-		~1		
		72	-		~5		
		96	-		~0.9		
					XS unit		
Soydawadawa (Soybean)	Boiled	0	6.59	ND	0.20	*Bacillus subtilis, Bacillus cereus, Bacillus pumilus, Bacillus licheniformis, Bacillus firmus*	(Opai-Tetteh, 1999)
		24	6.87		1.00		
		48	8.00		3.00		
		72	8.25		4.10		
	Roasted	0	6.41		0.30		
		24	6.57		1.10		
		48	7.90		2.45		
		72	8.15		3.60		
					U/g		

Table 2 (continued)

Product (Legume)	Processing conditions before fermentation	Fermentation time (h)	pH	Temperature (°C)	Proteolytic activity	Microorganisms identified/used	References
Ugba (African oil bean)	Boiled	0	5.0	Room temperature	~ 9	NI	(Njoku & Okemadu, 1989)
		12	5.5		~ 13		
		24	6.0		~ 27		
		36	6.3		~ 12		
		48	7.0		~ 11		
		60	7.2		~ 15		
		72	8.0		ND		
					U/mg/min		
Dawadawa (African locust bean)	Boiled	0	~ 6.0	ND	0	NI	(Zebedee et al., 2022)
		24	~ 6.25		~ 19		
		48	~ 6.9		~ 37		
		72	~ 7.5		~ 25		
		96	~ 8.5		~ 20		
					U/ml		
Iru (African locust bean)	Product obtained from the market	1		37	0.04	*Bacillus subtilis* PA2	(Olanbiwoninu., et al., 2022)
		1			0.05	*Bacillus licheniformis* PA8	
		1			0.12	*Bacillus subtilis* PA1	
		1			0.06	*Bacillus subtilis* PB1	
		1			0.03	*Bacillus licheniformis* PB7	
		1			0.08	*Bacillus subtilis* PB5	
		1			0.035	*Bacillus subtilis* PB6	
					U/ml		

Table 2 (continued)

Product (Legume)	Processing conditions before fermentation	Fermentation time (h)	pH	Temperature (°C)	Proteolytic activity	Microorganisms identified/used	References
Soybean-daddawa (Soybean)	Salted (1%) and salt-free daddawa			0	~ 500	*Bacillus subtilis* LB3 and *Staphylococcus xylosus* SAU3 (salted)	(Kolapo et al., 2023)
				24	~ 510		
				40	~ 511		
				65	~ 1000		
				0	~ 500	*Bacillus subtilis* LB3 and *Staphylococcus xylosus* SAU3 (salt-free)	
				24	~ 550		
				40	~ 650		
				65	~ 900		
				0	~ 390	*Bacillus subtilis* LB3 and *Leuconostoc mesenteroides ssp cremoris* (salted)	
				24	~ 500		
				40	~ 650		
				65	~ 650		
				0	~ 410	*Bacillus subtilis* LB3 and *Leuconostoc mesenteroides ssp cremoris* (salt-free)	
				24	~ 500		
				40	~ 510		
				65	~ 650		
				0	~ 650	*Staphylococcus xylosus* SAU3 and *Leuconostoc mesenteroides ssp cremoris* (salted)	
				24	~ 660		
				40	~ 750		
				65	~ 810		
				0	~ 500	*Staphylococcus xylosus* SAU3 and *Leuconostoc mesenteroides ssp cremoris* (salt-free)	
				24	~ 650		
				40	~ 750		
				65	~ 900		
				0	~ 550	*Bacillus subtilis* LB3 and *Staphylococcus xylosus* SAU3 and *Leuconostoc mesenteroides* spp *cremoris* (salted)	
				24	~ 560		
				40	~ 580		
				65	~ 800		
				0	~ 550	*Bacillus subtilis* LB3 and *Staphylococcus xylosus* SAU3 and *Leuconostoc mesenteroides* spp *cremoris* (salt-free)	
				24	~ 600		
				40	~ 610		
				65	~ 700		

ND Not determined, *NI* Not identified, *U/g* Units/gram, *U/ml* Units/milliliter, *mol/sec* Moles/second, *U-Unit. (U/ml)* A unit of activity produces a 0.01 absorbance unit difference at 420 nm between the sample and its blank assay, per minute, under the assay conditions (Oyedoh et al., 2020). (U/ml): One unit of protease activity was defined as the amount of enzyme required to release 1 µg of tyrosine per ml per minute under the mentioned assay conditions (Olajuyigbe & Ajele, 2008). (mol/sec): One unit of enzyme activity is defined as the amount of enzyme required to degrade a unit of albumin (mMol/s), under the reaction mixture conditions (Evans et al., 2009). (U) one unit of protease activity is defined as the amount of enzyme which released 1 mg of tyrosine from casein per minute under the assay conditions (Odibo et al., 2008). (XS unit): an enzyme extract which, under the stated experimental conditions, produced a filtrate with an optical density of 0.500 when measured in a 10 mm path length cell and a strength of 36 XS units per gram (Opai-Tetteh, 1999). (U/g): One unit of enzyme activity was defined as the amount of enzyme required to produce 1 pg leucine per min under the assay conditions (Njoku & Okemadu, 1989). (U/mg/min): One unit of protease activity is defined as the amount of enzyme which released 1 µg of tyrosine from casein per minute under the assay conditions (Zebedee et al., 2022). (U/ml): one unit of protease activity is defined as one micromole of substrate converted per minute (Olanbiwoninu., et al., 2022)

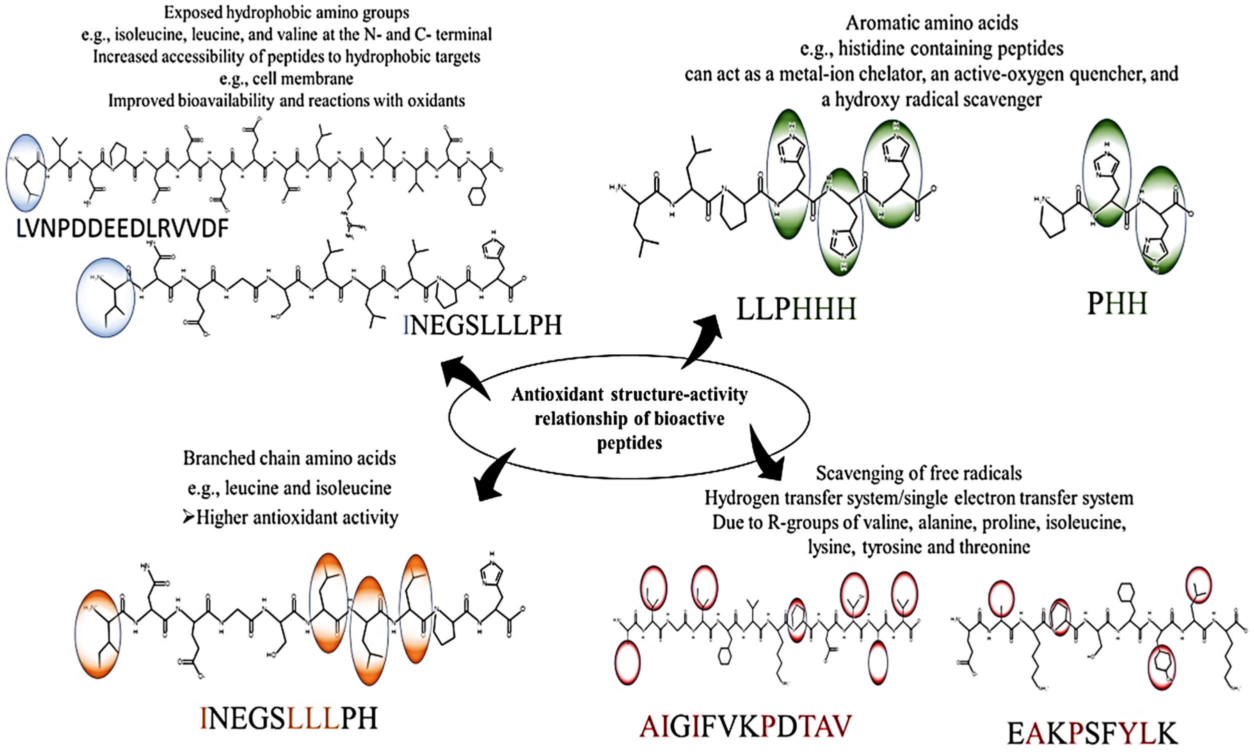


Fig. 3 A model illustrating the structure–activity relationship of antioxidant peptides

Structure–activity relationship of bioactive peptides and associated health benefits

Several studies have shown the benefits of fermentation in enhancing the bioactive properties of fermented legumes in Africa (Arueya et al., 2017; Kapravelou et al., 2014; Oboh et al., 2009). As previously mentioned, the bioactive properties of fermented legumes are largely associated with the presence of phenolic compounds. Microbial enzymes play a crucial role in releasing phenolics that are bound to plant cell walls, polysaccharides, and proteins, converting them into soluble forms with enhanced bioactivity. The fermentation process leads to the increased presence of aglycones, which are generated by microbial β-glucosidases and β-glucuronidases from phenolic glycosides, further contributing to the bioactive potential (Toor et al., 2021; Aganbi et al., 2023). As most of these legumes have high protein concentrations that could produce bioactive peptides, research focusing on the bioactivity of the produced peptides should be of interest in Africa. Researchers from other parts of the world have documented peptide health-promoting bioactivity from fermented legumes, such as the common bean, soybean, bitter bean, pea and red bean (Jakubczyk et al., 2017; Muhialdin et al., 2020; Vermeirssen et al., 2003; Wei et al., 2023; Xiao et al., 2018). Properties such as antioxidant activity, antihypertensive activity, anti-obesity, and anticancer activity have been documented in these fermented legumes. Some peptides have even been found to possess multifunctional activities, such that they may modulate more than one physiological outcome by affecting different targets (Lammi et al., 2019).

Antioxidant properties of peptides from fermented legumes

Antioxidant peptides may act as proton donors, metal ion chelators and radical scavengers (Fan et al., 2022; Xiong, 2010). These antioxidant activities rely on the molecular weight, composition, and sequence of amino acids in the peptide (Zou et al., 2016), as illustrated in Fig. 3. During protein hydrolysis, the unfolding of the folded globular structures of proteins increases the accessibility of the electron-dense peptide bonds and R-group (functional side chain) to the reactive species (Chai et al., 2020). Peptide R-groups such as valine, alanine, proline, histidine, isoleucine, leucine, phenylalanine, tryptophan, threonine and tyrosine may scavenge radicals via the hydrogen atom transfer (HAT) or the single electron transfer (SET) system (Chai et al., 2020; Fan et al., 2022). Tryptophan and tyrosine have indole and phenolic groups which can donate hydrogen atoms to reactive species (Pessione & Cirrincione, 2016). Hydrophobic amino groups (isoleucine, leucine, and valine), which are exposed at the N- and C-termini, can intensify the accessibility of peptides to hydrophobic targets such as the cell membrane, subsequently improving their bioavailability and reactions with oxidants (Chai et al., 2020). In addition to these structure–activity relationships, bioactive peptides from fermented legumes could also boost the body's antioxidant defense system via the activation of the kelch-like ECH-associated protein 1-NF-E2-related factor 2 (keap1-Nrf2) pathway (Fan et al., 2022; Wei et al., 2023).

Lentil alkaline fermentation with *L. plantarum* CECT 748 was found to inhibit reactive oxygen species (ROS) generation in tert-butyl-hydroperoxide (t-BOOH)-stressed macrophage-like, Abelson leukemia virus-transformed cell line (RAW 264.7) macrophages by approximately 50%, in contrast to nonfermented lentil, which had only 17.60% inhibition (Bautista-Expósito et al., 2018). The oxygen radical absorbance capacity (ORAC) value of the fermented lentil was 260.11 mM (Trolox equivalent) TE/g, while that of the unfermented lentil was 302.69 mM TE/g. However, lentils contain both peptides (99.62 mg/g) and phenolics (0.914 mg/g), which suggests that the bioactivities could be attributed to either compound. The type of peptide present might result in varying degrees of bioactivity in particular systems due to their structure–activity relationship. For example, a high concentration of hydrophobic peptides in the lentil extract may influence high solubility in RAW 264 cells, while they may not effectively scavenge peroxyl radicals through the hydrogen atom transfer process used in the ORAC system.

Peptides such as WMYNDQDIPVINNQLDQMPR DALEPDNRIESEGGLIETWNPNNRQ, FEEPQQSEQGEGR, RLNIGSSSSPDIYNPQAGR GSRQEEDEDEDE and RGEDEDDKEKRHSQKGES identified from the <3 kDa fraction of *L. plantarum* 299v-fermented faba bean (*Vicia faba*) seeds have also been found to exhibit antiradical activity (half-maximal inhibitory concentration (IC_{50}) = 0.02 mg/mL) (Jakubczyk et al., 2019). The presence of tryptophan and tyrosine in a peptide, e.g., WMYNDQDIPVINNQLDQMPR, can contribute to HAT reactions due to the indole and phenolic groups of the amino acids. R- groups of amino acids such as proline, isoleucine and valine could further influence the radical scavenging capacity of the peptide.

Anti-inflammatory activity of peptides from fermented legumes

Prolonged oxidative stress in the body can lead to chronic inflammation and oxidative damage of biomolecules. Inflammation occurs after inflammatory pathways are stimulated by inducing factors such as dextran sodium sulfate (DSS), lipopolysaccharide (LPS) and other toxicants, leading to the release of inflammatory markers (Liu et al., 2022). Peptides, particularly from soybean, have been shown to inhibit inflammatory markers such as tumour necrosis factor (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-β), nitric oxide (NO) and nitric oxide synthase (iNOS) in RAW 264.7 macrophages (Garcés-Rimón et al., 2022).

The size of a peptide can influence its anti-inflammatory activity. Low-molecular-weight peptides (<1 kDa) appear to possess high levels of anti-inflammatory activity due to their reduced enzymatic recognition and cleavage sites. This can facilitate their entry into the bloodstream as intact structures to exert anti-inflammatory effects on their target organs (Liu et al., 2022). A peptide chain containing at least one hydrophobic amino acid, such as leucine, tryptophan and phenylalanine, can induce anti-inflammatory properties (Liu et al., 2022). Peptides such as γ-glutamul-S-methylcysteine, γ-glutamul-leucine and XLe-Val-XLe, identified in the common bean (*Phaseolus vulga* L.) milk and yogurt, inhibited TNF-α-induced interleukin-8 (IL-8) in human colorectal adenocarcinoma (Caco-2) and human colon carcinoma (HT-29) cells (Chen et al., 2019). Other structural attributes of anti-inflammatory peptides include the presence of positively charged amino acids and the positioning of amino acids in the peptide chain (Liu et al., 2022).

Antihypertensive activity of peptides from fermented legumes

Peptides may regulate blood pressure by inhibiting enzymes (angiotensin-converting enzyme (ACE)) in the renin-angiotensin system (RAS) (Garcés-Rimón et al., 2022). The ACE can raise blood pressure by degrading bradykinin and by activating the conversion of angiotensin I to angiotensin II (Fan et al., 2022). Some food peptides have been found to reduce hypertension (high blood pressure) by inhibiting ACE enzymes. Structural characteristics such as chain length, composition and sequence can affect the ACE-inhibitory activities of peptides (Daskaya-Dikmen et al., 2017). Long-chain peptides containing aromatic tyrosine on the C-terminus can raise ACE-inhibitory activity. With regard to amino acid positioning and composition, tetrapeptides have their first, second and third amino acid positions occupied by tyrosine or cysteine, histidine, tryptophan, or methionine, and isoleucine, leucine, valine or methionine, respectively, which have known enhanced ACE-inhibitory activity (Daskaya-Dikmen et al., 2017). Peptide fractions (<1–3.5 kDa) from cooked tempe (*Rhizopus oligosporus*-fermented *Phaseolus lunatus* L.) had significantly ($P<0.05$) higher ACE inhibitory activity (84%) than 3.5–14 kDa fractions (81%) (Pertiwi et al., 2020). This indicates that the molecular weight (which is, in part, a

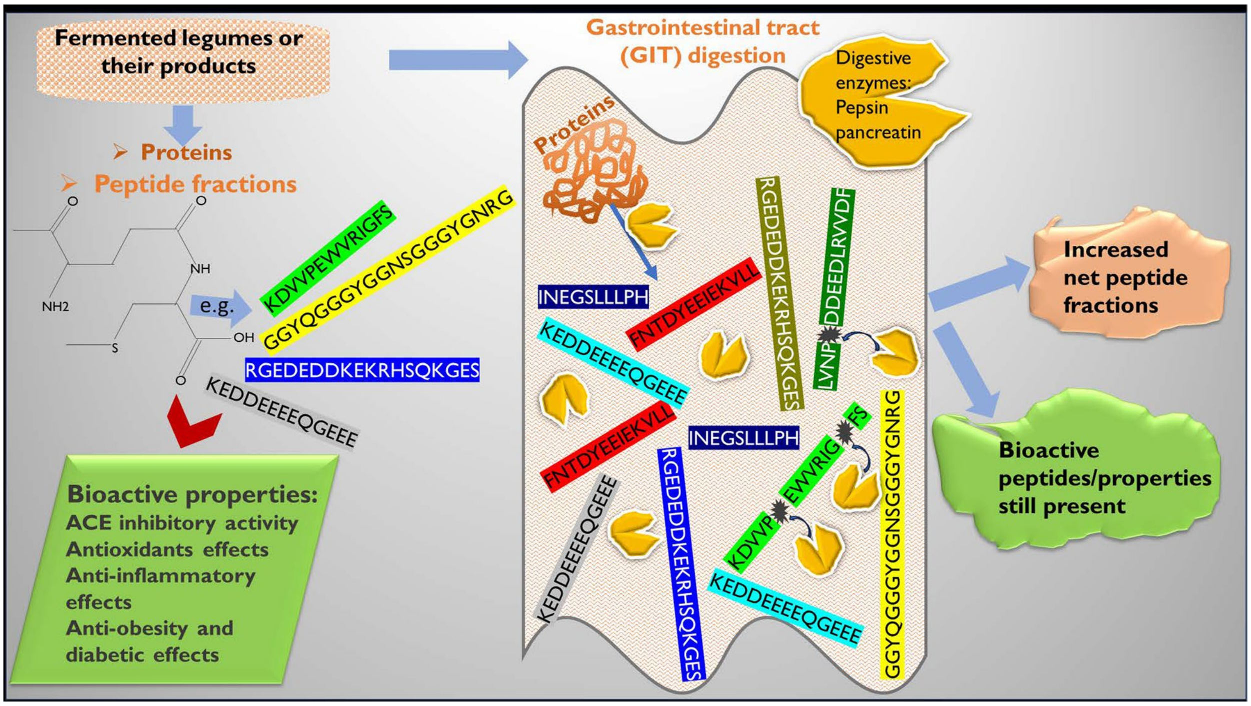


Fig. 4 A model illustrating the general stability/fate of bioactive peptides and/or bioactive properties after gastrointestinal tract (GIT) digestion of fermented legumes

function of the peptide chain or composition) may have influenced the ACE-inhibitory activity.

Stability of bioactive peptides from fermented legumes after GIT digestion

The stability and bioaccessibility of bioactive peptides in the GIT are crucial in ensuring their absorption into the bloodstream and utilization in the target tissues or organs which affects their functional effect or bioactivity as health-promoting foods (Indrati, 2021).

On food consumption, peptides from foods need to resist digestive enzymatic actions in the GIT and cross the intestinal epithelial barrier to reach the target organs in an intact and active form to exert their health-promoting effects (Amigo & Hernández-Ledesma, 2020). Gastrointestinal digestion of foods, including fermented food, can confer additional chemical modifications to food proteins, and may facilitate the release of new bioactive peptides and/or degrade the existing peptides (Bautista-Expósito et al., 2018), as illustrated in Fig. 4. GIT digestion has been proposed to be a dominant factor controlling the formation of bioactive peptides, notably ACE inhibitory peptides (Indrati, 2021; Sánchez-García et al., 2024; Vermeirssen et al., 2003). However, GIT proteolytic actions can also result in the loss of amino acid residues, which will change peptide activities, as the potential bioactive properties of a peptide also depend on its amino acid composition (Verni et al., 2019). In mitigation, it has been reported that peptides with small molecules, as well as some higher molecular weight peptides, can be absorbed from the GIT without further digestion, which makes amino acid composition a key factor in digestion resistance in the GIT.

Research regarding the fate (stability/bioaccessibility) of bioactive peptides in African fermented legumes in the GIT is limited, although some studies have focused on fermented legumes and their products in general, such as fermented black beans, common beans, faba beans, lentil seeds, lima beans, and pea seeds (Table 3), many of which are consumed in Africa. These studies found that the peptide contents of the digested fermented legumes were substantially increased after GIT digestion, as illustrated in Fig. 4. This increase was attributed to the formation of new peptides by GIT proteases and peptidases (Bautista-Expósito et al., 2018) as well as an improved degree of protein hydrolysis, especially at the pancreatic stage, which can also be enhanced by fermentation (Pertiwi et al., 2020; Wang et al., 2022). Table 3 shows that after undergoing GIT digestion, the fermented legume product digests (or their peptide fractions, particularly those < 3–10 kDa) exhibited health-promoting properties/bioactivities in vitro, including ACE inhibitory activity, ORAC, ROS, anti-obesity activity and α-amylase inhibitory activity. Pertiwi et al. (2020) revealed that the

Table 3 Stability and/or bioaccessibility of bioactive peptides and/or bioactive properties after simulated in vitro gastrointestinal tract (GIT) digestion of fermented legumes

Legumes or their extracts or products	Microorganism/ enzymes	Fermentation conditions (pH, temperature, time)	GIT enzymes used	Bioactive properties after GIT digestion	Important associated peptides and their parent protein and peptide molecular mass	References
Lentils seeds	*Lactobacillus plantarum* with Savinase	8.5; 37 °C; 15 h	Pepsin, pancreatin	Angiotensin -I- converting enzyme (ACE) inhibitory activity (IC_{50} 0.23 mg/mL), α-sucrase inhibitory activity (39%) was present but reduced, antioxidant activity (oxygen radical absorbance capacity (ORAC)); increased radical oxygen species (ROS) production inhibition; maltase activity and lipase inhibition were lost	SGREKWERKEDEEKVVEEEEGEWRGS, KDVVPEWVRIGFS, LNTKYDTIEKVLLEEQENEPH, LVNPDDEEDLRVVDF, SLNTKYDTIEKVLLEQENEPH, FNTDYEEIEKVLL, FNTEYEEIEKVLLEEQEQKSQ, KDVVPEWVRIGFSA and more. Peptide sequence derived from convicilin, vicilin (allergen Len c 1.0101 and 1.0102) and lectin 1.1–3.2 kDa	(Bautista-Expósito et al., 2018)
Lentils (Pardina and Castellana) seeds and flour (Lens culinaris)	Pleurotus ostreatus	pH not reported; 28 °C; 14 days	Amylase, pepsin, pancreatin-bile salts	ACE inhibitory activity (%) was present and substantially increased (from approx. 8–15% up to 70%) but was similar or lower than unfermented products, indicating that the net inhibitory activity derived during fermentation was lost	Associated peptides not identified 0.45 kDa, 1.4 kDa and ≥ 12.5 kDa	(Sánchez-García et al., 2024)
Bean seeds (*Phaseolus vulgaris* L.) (dehulled)	*Lactobacillus plantarum 299v*	pH not reported; 22 °C, 30 °C and 37 °C; 3 h, 3 days and 7 days	α-Amylase, pepsin, pancreatin	ACE inhibitory activity (IC_{50} 0.28–7.65 mg/mL), and anti-lipase inhibitory activity (1.19–6.25 mg/mL) were present depending on fermentation conditions	INEGSLLLPH, FVVAEQAGNEEGFE, SGGGGGGVAGA ATASR, GSGGGGGGGFGGPRR, GGYQGGGYGGNSGGGYGNRG, GGSGGGGGSSSGRRP, GDTVTVEFDTFLSR. Peptide sequence derived from vicilin, legumin J-like 3.5–7.0 kDa	(Jakubczyk et al., 2017)
Lima beans (*Phaseolus lunatus* L.)	*Rhizopus oligosporus*	pH not reported; room temperature; 36 h soaking before 48 h fermentation	Pepsin, pancreatin	ACE inhibitory activity was present and was slightly increased (up to IC_{50} 0.59 mg/mL)	Associated peptides not identified < 1 kDa (majorly), 1–3 kDa, 3.5–14 kDa	(Pertiwi et al., 2020)

Table 3 (continued)

Legumes or their extracts or products	Microorganism/ enzymes	Fermentation conditions (pH, temperature, time)	GIT enzymes used	Bioactive properties after GIT digestion	Important associated peptides and their parent protein and peptide molecular mass	References
Faba bean seeds (*Vicia faba* L. var.)	*Lactobacillus plantarum 299v*	pH not reported; 22 °C, 30 °C and 37 °C; 12 h soaking before 3 h, 3 days and 7 days fermentation	α-Amylase, pepsin, pancreatin	Antiradical activity against ABTS (IC_{50} 3.51–0.99 mg/mL), ACE (IC_{50} 2.9–1.01 mg/mL), Lipoxidase (IC_{50} 2.90–0.54 mg/mL) and lipase (IC_{50} 2.31–0.89 mg/mL) inhibitory activities were present depending on the fermentation conditions	DALEPDNRIESEGGLIETWNPNNRQ, FEEPQQSEQGEGR, GSRQEEDEDEDE, WMYNDQDIPVINNQLDQMPR, RGEDEDDKEKRHSQKGES, RLNIGSSSSPDIYNPQAGR. Peptide sequence derived from N-terminal legumin A1 prepro-polypeptide	(Jakubczyk et al., 2019)
Common bean (*Phaseolus vulga* L.) milk and yogurt	*Bifidobacterium bifidum*, *Lactobacillus spp*, *Streptococcus thermophilus*	Up to 4.5; room temperature, 4–14 h	Pepsin, pancreatin	Cellular antioxidant activity (peroxyl radicals quenching ability) (IC_{50} 0.1–0.5 mg/mL), and cellular anti-inflammatory activity, i.e., inhibition of IL-8 secretion induced by TNF-α were present	γ-glutamyl-S-methylcysteine, γ-glutamyl-leucine, Xle-Val-Xle < 10 kDa and 10–50 kDa	(Chen et al., 2019)
Black bean tempeh	R. oligosporus	pH not reported; 35 °C; 39 h	Amylase, pepsin, trypsin	ACE inhibitory activity (%) was present and substantially increased (from approx. 30% up to 83%) but was lower than unfermented products indicating that some ACE inhibitory activity was lost due to fermentation	Associated peptides not identified < 10 kDa and ≤ 22 kDa	(Wang et al. ,2022)
Pea seeds (*Pisum sativum* var. Bajka) protein	*Lactobacillus plantarum 299v*	pH not reported; 22 °C, 30 °C and 37 °C; 12 h soaking before 3 h, 3 days and 7 days fermentation	α-Amylase, pepsin, pancreatin	ACE inhibitory activity was present (IC_{50} 0.48–0.19 mg/mL). Further isolation of peptide fractions yielded lower IC_{50} values of 0.064–0.14 mg/mL	KEDDEEEEQGEEE, parent protein not reported. < 1.6 kDa and < 7 kDa	(Jakubczyk et al., 2013)

Table 3 (continued)

Legumes or their extracts or products	Microorganism/enzymes	Fermentation conditions (pH, temperature, time)	GIT enzymes used	Bioactive properties after GIT digestion	Important associated peptides and their parent protein and peptide molecular mass	References
Pea protein isolates	*Lactobacillus helveticus* and/or *Saccharomyces cerevisiae*	6.1–3.5; 37 °C; 48 h	Pepsin, trypsin, α-chymotrypsin	ACE inhibitory activity was present (IC_{50} 0.11–0.23 mg/mL) but similar to unfermented peas, suggesting that the net ACE inhibitory activity derived during fermentation was lost	Associated peptides not identified 3 kDa	(Vermeirssen et al., 2003)

percentage of ACE inhibitory activity increased slightly after GIT digestion of fermented lima beans, attributed to the possible formation of new ACE inhibitory peptides during digestion. A more advanced in vitro technique using Caco-2 cells and/or HT-29 cells also showed that fermented common bean product digestate and peptide fractions, particularly those < 10 kDa, exhibited antioxidant activity, the ability to quench peroxyl radicals in the cell surface membrane and anti-inflammatory activity, as well as the inhibition of IL-8 secretion induced by TNF-α after treating the cells (Chen et al., 2019). An in vivo absorption study in male rats revealed that a significant portion of peptides from fermented lima bean products can be successfully absorbed into different segments of the small intestine (duodenum, jejunum and ileum) (Pertiwi et al., 2020). The absorbed peptides exhibited ACE inhibitory activity, indicating a significant presence of ACE inhibitory peptides after the absorption process. Overall, these findings suggest that bioactive peptides exhibiting the above-mentioned bioactive activities can be stable or bioaccessible after GIT processes.

Jakubczyk et al. (2013, 2017, 2019) and Vermeirssen et al. (2003) showed that the stability or bioaccessibility of the bioactive peptides of fermented legumes could be dependent on fermentation conditions such as temperature and time, as well as the microbial strains used during fermentation (Table 3). The potential health-promoting properties of the fermented legume products or peptides after GIT digestion varied significantly with these conditions. The likely mechanism for this effect would be worth exploring in future research to better understand how these fermentation conditions affect the stability/bioaccessibility of bioactive peptides.

Bautista-Expósito et al. (2018), Vermeirssen et al. (2003) and Sánchez-García et al. (2024) all showed that the bioactivity of peptides, such as ACE inhibitory peptides or activity obtained due to fermentation, could be reduced or lost after GIT digestion (Table 3), likely due to degradation of ACE inhibitory peptides by gastrointestinal proteases. Although ACE inhibitory activity (%) significantly increased after GIT digestion for both fermented and unfermented legume products, such as in black bean tempeh, lentils seeds and flour and pea protein isolates, the ACE inhibitory activity of the fermented products was similar and/or lower than in the unfermented products, and the unfermented products were at least as ACE inhibitory active as the fermented ones. The authors of these studies concluded that the similar ACE inhibitory activity in fermented pea protein isolates after GIT digestion compared to the unfermented ones was likely due to the adverse effect of microbial enzymes split within the bioactive peptide sequence in the food protein, consequently preventing their release by GIT proteases. There appears to be an optimal degree of hydrolysis above which more ACE inhibitory peptides are degraded than new peptides are formed, which decreases the overall ACE inhibitory activity. However, the IC_{50} value of the fermented product was not quantified before GIT digestion in these studies, and so it is difficult to determine whether there was, in fact, an initial improvement in the efficacy of ACE inhibitory activity due to the investigated fermentation conditions in comparison to the unfermented product. Bautista-Expósito et al. (2018) did not show the IC_{50} value of the unfermented products after GIT digestion, and so it is unclear whether additional peptide bioactivity derived from the investigated fermentation technology was lost or was only reduced. However, Wang et al. (2022) did show that fermentation of black beans (with *R. oligosporus*) had initially substantially reduced the ACE inhibitory activity prior to GIT digestion, suggesting that fermentation conditions may play a role.

Overall, the data from the reviewed studies suggest that a substantial portion of the bioactive peptides from fermented legumes can be stable and bioaccessible after GIT digestion and can potentially reach their target organs in the body to exert their health-promoting effects. The stability or bioaccessibility of bioactive peptides from fermented legumes after GIT digestion merits further, well-designed research to validate these findings, as the GIT digestion reported in these studies was mostly measured by an in vitro simulated GIT model. Although in vitro assays are important in the investigation of bioactive peptides and their stability or bioaccessibility, more effort should be made to validate their in vivo bioaccessibility.

Conclusions, recommendations and future perspectives

Fermentation plays a key role in the development of functional foods, especially in Africa, where a variety of protein-rich legumes are abundant. Legumes such as the cowpea, African yam bean and bambara groundnut have been traditionally fermented, either spontaneously or through controlled processes. Research indicates that peptides produced during fermentation may possess beneficial bioactivities, including antioxidation, anti-inflammation and antihypertensive effects. Further investigation is needed to fully understand the bioactivities of peptides from fermented legumes and to optimize fermentation conditions. Factors such as pH, temperature, and nutrient availability can be adjusted to enhance peptide production. The stability of these peptides during processing and gastrointestinal digestion requires scrutiny to ensure their bioactivity post-absorption. Legume cultivation and fermentation can contribute to sustainable environmental practices and processing techniques, potentially reducing the incidence of non-communicable diseases related to oxidative stress and inflammation. Functional legume products derived from fermentation hold promise as sources of bioactive compounds beneficial for human health.

Acknowledgements

The authors would like to thank Dr James Elegbelye, Tosin Olaniran and Memory Muhau from the University of Pretoria for providing pictures of fermented African locust bean and cowpea in Fig. 1.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used Grammarly and ChatGPT to correct the English grammar. After using this tool/service, the author(s) reviewed and used the services of a professional English proofreader. Therefore, they take full responsibility for the content of the publication.

Authors' contributions

Conceptualization, SMM and EK.; writing and original draft preparation, SMM and OYF.; writing review and editing, SMM, OYF and EK.; project administration, EK.; funding acquisition, EK.

Funding

This work was supported by the University of Pretoria for SM. Moyo and OY Famuyide under the UP postdoctoral fellowship programme. Grant Source: DRI—Cost Centre: A0X 816.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have approved the manuscript for submission.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Received: 17 November 2023 Accepted: 12 April 2024

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