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Antibacterial Metabolites Obtained from Fermentation of Peanut and Cowpea by *Lactobacillus* spp

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ABSTRACT: Lactic acid bacteria elective habitat is food matrix, where they release encrypted metabolites from several parent proteins as a result of their proteolytic activity in the matrix. These metabolites when decrypted confer different bioactive activity thus improving public health. This study was aimed at producing peptide containing metabolites with antibacterial efficacy from defatted cowpea and peanut using *Lactobacillus* spp. *Lactobacillus* spp. isolated from spoilt yoghurt were identified using culture dependent and independent method. The isolates were screened for proteolytic ability on skimmed milk agar. The selected isolate with highest proteolytic activity was used for metabolites production through fermentation of defatted cowpea and peanut at 37 ºC for 72 h. Parameters analyzed during fermentation were pH, *Lactobacillus* count, protease activity, peptide concentration and antibacterial activity. The crude peptides produced were assayed for antibacterial activity against bacteria isolated from spoilt meat. From the identified *Lactobacillus* spp., *L. plantarum* CAU4347 had the highest proteolytic activity with clear zone of 24.50 ± 0.707 mm. During fermentation the highest and lowest *Lactobacillus* counts were from cowpea and peanut media with values 294.2 ± 0.21 $\times 10^7$ and 0.60 ± 0.4 $\times 10^7$ cfu/ml respectively. Cowpea medium inoculated with *L. plantarum* CAU4347, had the highest peptide concentration of 79.92 ± 0.01 µg/ml. Consequently, peanut medium showed higher antibacterial activity of 18 mm against *Escherichia coli*. This result finding suggests that encrypted peptide metabolites from cowpea and peanut flour can confer antibacterial activity against meat spoilage bacteria thus could be utilized as a potential bio-preservative.

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Food preservation principle is mainly concerned with preventing microbial spoilage that will result to several negative changes in integrity and loss of nutritional value of food (Rawat, 2015). To suppress the growth and survival of these microorganisms, a process which is dependent on microbial biological activity for metabolites production is used. This is an old method utilized for food and beverage preservation and production thousands of years ago, known as fermentation. Different food varieties have been used by yeast and lactic acid bacteria, in both cases the food acts as substrate for the release of microbial metabolites which contribute to its shelf life extension (Korhonen and Philanto, 2006). Likewise, during bacteria or yeast growth in a protein material medium, enzyme secretion (especially proteases) hydrolyzes the parent protein to release peptides which confer different bioactive activity in their crude and pure forms. The fermentation product can be used directly or could be centrifuged to obtain peptide containing metabolites with lengthy chains and wide array of amino acid compositions (Jakubczyk *et al*., 2013). Peanut and cowpea are known to have innate beneficial bioactive compounds inherent in their parent protein thus ascribing physiological roles when released (Udenigwe and Aluko, 2012). Asides the nutritional constituents of these legumes, they also contain anti-nutrients which interfere with protein digestibility. These anti-nutrients can be detoxified by dehulling, soaking, boiling, steaming, roasting, sprouting and fermentation treatment (Phulia *et al*., 2018; Nwafor *et al*., 2017), leaving behind nutritional compositions for microbial protease hydrolysis. These proteases endogenously or exogenously attack sited small amino or carboxyl group of parent protein (Demirci *et al*., 2014) to release peptides. These peptides can be applied as functional food additives, animal feed, infant food formulations, fortifier for fruit juice and soft drink as well as specific therapeutic food products (Balakrishnan *et al*., 2011); or allowed to pass through costly downstream purification process. The high value hydrolyzate containing peptides also exhibited antioxidant, antimicrobial, antihypertensive, anticarcinogenic, immunomodulatory and antithrombotic activities (Hartmann and Meisel, 2007; Chalamaiah *et al*., 2012; Agyei and Danquah, 2012). Research has revealed crude and pure antimicrobial peptides (AMPs) obtained from soybean, which

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reflects that plant proteins are good source of potential bio-preservative (Biobaku *et al*., 2017; Dhayakaran *et al*., 2016). Their activity depends on the protein type used, extent of hydrolysis and the amino acid sequences in the hydrolyzate (Alvarez-Ordonez *et al*., 2013). The aforementioned hydrolyzate can be formed by the hydrolytic action of lactic acid bacteria (LAB) derived protease on proteins produced by cowpea and peanuts such as globulins, albumins, arachin and coarachin (Osman *et al*., 2013; Abdel-Shafi *et al*., 2019) to increase protein content, remove anti-nutrients content (Adeyemo and Onilude, 2013) and release peptides of bioactive ability (Mohanty *et al*., 2016). These organisms have displayed impressive attributes as starter culture in peptide assay. They are cheaper, have rapid accelerated metabolic activities, improved safety, reduced hygienic risks and toxigenic risk, shown high viability and survival prowess (Holzapfel, 2002). Species that have been implicated in antimicrobial peptide production are *Lactobacillus rhamnosus, L. plantarum, L. acidophilus, L. delbreuckii L. paracasei, L. pentosus* (Rana and Bajaj, 2015), *Bacillus subtilis* NB22 and *Virgibacillus* sp. (Lapsongphon and Yongsawatdigul, 2013). This study was to investigate the antibacterial activity of metabolites produced by *Lactobacillus* sp. during fermentation of cowpea and peanut.

MATERIALS AND METHOD

Sample Collection and Preparations: Peanut and cowpea purchased from an open market in Benin City were prepared by soaking 3 kg of the grains in 6 L of distilled water for 15 min and dehulled by crushing in-between the palms. Then sun-dried for 3 days and ground into powdered form using a hand blender. The powder samples were stored in clean bottles for further laboratory analysis. From each finely ground sample 1000 g was weighed and mixed with 5 L analytical n-hexane in a Soxhlet apparatus for extraction. After 24 h of extraction, by constantly recycling 1000ml of the solvent, the defatted samples were oven dried (Lemfield Medical England, Model DHG) at 40 ^{o}C for 4 h and allowed to cool (Liu and Chiang, 2008).

Proximate Analysis: The protein, fat, carbohydrate, ash, moisture and fiber contents of dehulled defatted cowpea and peanut samples were determined by the methods of AOAC (1990).

Isolation and characterization of Lactobacillus spp.*:* Fresh yoghurt was allowed to undergo spoilage after which 10 fold serially dilution was carried out using sterile distilled water as a diluent. An aliquot of the appropriate dilution was spread plated on De Man Rogosa Sharpe agar (MRS) (Oxoid, UK) and incubated at 37 ^{o}C for 48 h. Colonies of interest were identified based on cultural characteristics observed on agar plates and sub-cultured into MRS medium, purified on nutrient agar and transferred to slants. Presumptive selected biochemical screening was carried out on purified colony of selected bacterial isolates sub-cultured on nutrient agar. DNA of the single colony purified on nutrient agar was extracted by following the instruction on the manufacturer's manual on the DNA extraction kits. And pure DNA was amplified using the polymerase chain reaction. The amplified fragments were sequenced (Genetic Analyzer 3130xl sequencer) from Applied Bio-systems using sequencing kit (Big Dye Terminator v3.1 Cycle Sequencing kit). Bio-Edit software and MEGA 6 were used for all genetic analysis (Cheesbrough, 2000; Da Trindade *et al*., 2007).

Screening of Lactobacillus Isolates for Proteolytic Activity: An inoculum of a 24 h old culture of *Lactobacillus* isolates on nutrient agar was transferred into MRS broth in test tubes and incubated at 37 ^{o}C for 48 h. Then 10^{8} cfu/ml suspension was prepared which is equivalent to 0.5 McFarland. And 0.5 ml of the suspension was infused in holes bored (with 6mm diameter cork borer) on skim milk agar (1% skim milk, 0.5 % peptone, 0.3 % yeast extract, 15 % agar and 0.5 % NaCl) plate and incubated at 28±2 ^{o}C for 24 h. After which the zone of clearance was recorded. The highest zone of clearance indicated high proteolytic activity on skim milk. The *Lactobacillus* isolate with the highest proteolytic activity was selected for metabolite production.

Inoculum Preparation: The screened *Lactobacillus* isolate with high proteolytic activity was cultured on MRS agar at 37 ^{o}C for 24 h. Cell pellet free from MRS medium was obtained and transferred to a test tube containing 2 ml sterile saline to prepare 10^{8} cfu/ml inoculum suspensions. The saline containing tube was vortexed to make smooth suspension and the turbidity of each suspension was compared with 0.5 McFarland standards whose absorbance at 625nm should be 0.08 to 0.10, equivalent to 10^{8} cfu/ml.

Fermentation Experiment: From the defatted peanut and cowpea samples 80 g were weighed into separate 1000 ml conical flasks containing 720 ml distilled water. The pH of the mixture was stabilized to 6.4 using 0.1 N NaOH and/or HCl, then autoclaved at 121 °C for 15 min. The sterile broth was inoculated with 2 ml suspension of the screened *Lactobacillus* isolate (equivalent to 1.5×10^{8} cfu/ml) and fermented at 37 °C for 72 h. Samples were analyzed at every 24 h interval for change in pH, Lactobacilli count, protease activity, peptide concentration and anti-bacterial activity (Singh *et al*., 2015).

Viable Lactobacilli Count: Lactobacillus count of the fermented samples was carried out using 10-fold serial dilution and spread plate method. An aliquot of 100µl each was transferred into plate containing De Man Rogosa Sharpe agar (MRS, Oxoid, UK.) and incubated at 37 ^{o}C for 48 h, after which colonies were counted and recorded as cfu/ml (Park *et al*., 2012).

pH and Protease Activity Determination: The pH was determined using pH meter. The protease activity of fermented samples was determined using the azocasein method and absorbance measured at 440nm (Slivinski *et al*., 2012).

Peptide Extraction and Quantification: Each fermented sample was centrifuged with 10ml centrifuged tubes at 14000 rpm for 15 mins and the supernatant suspension was adjusted to pH 7.0 using 1M NaOH to exclude the antimicrobial effects of organic acid, followed by filtration of the supernatant. Inhibition activity from hydrogen peroxide (H_2O_2) was eliminated by the addition of 5 mg/ml catalase and freeze dried at -40^{o}C. Peptide quantification was determined on the crude freeze dried peptide samples using the ortho-phthaldehyde (OPA) assay method (Wang *et al*., 2008) and the absorbance measured spectrophotometrically at 340 nm, after 5 min at 28±2 ^{o}C. The standard serine curve was determined using serine concentration range of 25-250 µg/ml and the peptide concentration was estimated by extrapolation from the standard curve.

Antibacterial Activity**:** The test organisms used for antibacterial activity of crude metabolites were *Bacillus cereus*, *Salmonella enteritidis*, *Enterobacter cloacae*, *Klebsiella variicola*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. The test organisms were collected from the Department of Microbiology Laboratory, University of Benin, Benin City and confirmed using culture based method (Cheesbrough, 2000). To determine the antibacterial activity (using agar well diffusion method) of peptides, 50 mg/ml crude freeze dried peptide sample was prepared and 1000 µl of it was infused into wells carried on Mueller Hinton agar containing plates with a cork borer of 6 mm in diameter.

These plates have been prepared by inoculating with 100 µl broth containing each test organism (grown overnight at 37 ^{o}C and standardized to 10^{8} cfu/ml using McFarland methods) using the spread plate method. Then the zone of inhibition was measured after 24 h of incubation in mm and ciprofloxacin was used as positive control.

Statistical Analysis: During this experiment all data obtained were subjected to variance analysis at $P<0.05$ using SPSS 15.0 software package. Duncan's New Multiple Range Test was used to separate means (Ogbeibu, 2005).

RESULTS AND DISCUSSION

The nutritional composition of dehulled defatted peanut and cowpea meal is presented in Table 1. From the composition, cowpea had the highest carbohydrate content of 52.92 ± 1.36 % followed by protein content of 28.59 ± 0.77 % and the least 2.00 ± 0.00 % was crude fat. For peanut, the highest composition was protein content followed by carbohydrate content and the least was crude fat, with values of 49.29 ± 0.77 %, 29.37 ± 1.95 % and 1.33 ± 0.33 % respectively. The carbohydrate concentration of both samples, serve as carbon and energy sources required for cell growth (Godbey, 2014). Proteinous foods are sources of encrypted biopeptides and amino groups released through enzyme hydrolysis or fermentation which confer different bioactive activity (Aluko, 2015). Higher protein and lower carbohydrate content observed in defatted peanut sample correlate with studies carried out by Khalid and Elhardallou, (2013) who observed that the protein content of defatted cowpea flour is 26.73 %, which is lower than that of defatted peanut (55.88 %) (Wu *et al*., 2009) Biochemical and molecular characterization of the *Lactobacillus* spp. isolated from spoilt yoghurt is shown in Table 2.

Table 1: Nutritional composition of defatted legume seeds

Chemical analysis	Cowpea	Peanut
Moisture content (%)	7.33 ± 1.30	8.67 ± 1.67
Ash content (%)	5.67 ± 0.33	10.00 ± 1.16
Crude fat (%)	2.00 ± 0.00	1.33 ± 0.33
Crude fibre (%)	3.50 ± 0.29	1.33 ± 0.17
Protein content (%)	28.59 ± 0.77	49.29 ± 0.77
Carbohydrate content (%)	52.92 ± 1.36	29.37 ± 1.95

Legend: Values are mean ± standard deviation of triplicate.

The identified isolates coded L5, L7, L9, L11 were 99 % similar to *Lactobacillus plantarum* CAU4347, *Lactobacillus pentosus* NRIC 1557, *Lactobacillus plantarum* SNK12 and *Lactobacillus plantarum* L142 respectively. Isolated *Lactobacillus* spp. displayed diverse level of zone of clearance (mm) on skimmed milk agar which is indicative of proteolytic ability as shown in Table 3. The highest zone of clearance of 24.50 ± 0.71 mm was produced by *L. plantarum* CAU4347 while the least of 19.00 ± 1.41 mm was produced by *L. plantarum* SNK12.

Table 2: Biochemical and molecular characterization using 16S rRNA gene of *Lactobacillus* from yoghurt

Isolate code	Gram reaction	Catalase test	Spore formation	Identity (%)	Accession number	Confirmed strains
L5	+	-	-	99	MF424125.1	*Lactobacillus plantarum* CAU4347
L7	+	-	-	99	AB362714.1	*Lactobacillus pentosus* NRIC 1557
L9	+	-	-	99	KX426268.1	*Lactobacillus plantarum* SNK12
L11	+	-	-	99	JQB01723.1	*Lactobacillus plantarum* L142

The proteolytic activity of *Lactobacillus* spp. have revealed the ability of all isolates to secrete extracellular proteolytic enzymes by being able to hydrolyze casein molecules into colourless peptide fragments as reflected by the clear zone around each culture. Pailin *et al*. (2001) conducted a similar study and reported all LAB isolates to produce high zone of clearance on skim milk. In this study, *Lactobacillus plantarum* CAU4347 had the highest zone of clearance. This isolate can be an efficient and high yielding proteolytic LAB that could have the potential of liberating bioactive peptides from parent proteins (Virtanum *et al*., 2007). Various authors have reported that *L. plantarum* has a high proteolytic activity (Toe *et al*., 2019).

Table 3: Zone of clearance (mm) on skimmed milk agar using the isolated *Lactobacillus* spp.

***Lactobacillus* spp.**	**Zone of clearance**
Lactobacillus plantarum CAU4347	24.50 ± 0.71
Lactobacillus pentosus NRIC 1557	21.50 ± 1.41
Lactobacillus plantarum SNK12	19.00 ± 1.41
Lactobacillus plantarum L142	20.00 ± 0.71

Values are mean ± standard deviation of duplicate.

L. plantarum CAU4347 was selected for production of antibacterial metabolites through the fermentation of defatted cowpea and peanut proteins. The change in pH during *Lactobacillus plantarum* CAU4347 fermentation of peanut and cowpea is shown in Table 4. There was a decrease in pH as fermentation time progressed. The lowest pH at 72 h of fermentation was 5.19 ± 0.18 and 5.36 ± 0.33 in the peanut and cowpea media respectively. Table 5 showed the viable *Lactobacillus* count during fermentation of peanut and cowpea. There was a progressive increase of Lactobacillus cell counts during the fermentation period. At 72 h of fermentation, the highest Lactobacillus count was 294.2 ± 0.21 $\times 10^7$cfu/ml from cowpea medium, while the least was 180.81 ± 0.13 $\times 10^7$cfu/ml from peanut medium. The significant decrease in pH, is attributed to lactic acid produced, thus causing an increase in the acidity of the medium (Thu *et al*., 2011). Singh and co-workers (2015) observed increase in acidic level as the fermentation time increases during the production of antimicrobial peptides from soy milk protein by *Lactobacillus* spp. On the contrary, the increase in growth of *L. plantarum* CAU4347 could be as a result of difference in medium composition, which has a number of factors that affect growth and functionality of LAB (Abbasiliasi *et al*., 2017). The highest *L. plantarum* CAU4347 growth observed in cowpea medium after 72 h of fermentation could be as a result of the presence of sufficient carbon and energy source in the medium, utilizable for growth over a longer period of time than its lower carbohydrate containing medium counterpart. These observations aligned with Rana and Bajaj's (2015) research findings, which shows an increase in *L. acidophilus* and *L. delbrueckii* from 14 h to 48 h.

Table 4: Changes in pH during fermentation of defatted legume seeds

Fermentation Time(h)	**Peanut**		**Cowpea**		***P*-value**
	Control	***L. plantarum***	**Control**	***L. plantarum***	
0	6.43 ± 0.03^a	6.40 ± 0.06^a	6.40 ± 0.06^a	6.40 ± 0.06^a	0.958
24	6.39 ± 0.03^a	5.65 ± 0.13^b	6.33 ± 0.03^a	5.91 ± 0.11^b	0.002
48	6.37 ± 0.08^a	5.46 ± 0.3^b	6.28 ± 0.07^a	5.68 ± 0.04^b	0.013
72	6.35 ± 0.06^a	5.19 ± 0.18^b	6.30 ± 0.02^a	5.36 ± 0.33^b	0.006

Legend: *Values are mean ± standard error of triplicate. Same superscript alphabets across the row mean statistically not significant while those with different superscript means statistically significant (p<0.05)*

Table 5: *Lactobacillus* counts ($\times 10^7$cfu/ml) during fermentation of defatted legume seeds

Fermentation Time(h)	**Peanut**		**Cowpea**		***P*-value**
	Control	***L. plantarum***	**Control**	***L. plantarum***	
0	0.60 ± 0.4^a	1.50 ± 0.5^b	0.60 ± 0.4^a	1.70 ± 0.3^b	0.001
24	5.00 ± 0.4^a	136.62 ± 0.11^b	3.45 ± 0.26^a	282.7 ± 0.23^c	0.000
48	9.80 ± 0.72^a	147.6 ± 0.17^b	4.50 ± 0.35^a	206.3 ± 0.19^c	0.000
72	11.00 ± 0.17^a	180.81 ± 0.13^b	8.60 ± 0.11^a	294.2 ± 0.21^c	0.000

Legend: *Values are mean ± standard error of triplicate. Same superscript alphabets across the row mean statistically not significant while those with different superscript means statistically significant (p<0.05).*

Protease activity during fermentation of defatted cowpea and peanut by *L. plantarum* CAU 4347 is presented in Figure 2. The highest protease activity was observed at 24 h of fermentation with values of 80.56 ± 1.10 and 125.88 ± 1.7 for peanut and cowpea medium respectively. The least protease activity was observed at 72 h of fermentation with values of 4.16 ± 0.09 U/ml and 13.09 ± 0.17 U/ml for peanut and cowpea media respectively. Shown in Figure 3 is the concentration of peptides produced when cowpea and peanut media were fermented by *L. plantarum* CAU 4347 for 72 h. At 48 h of fermentation the highest peptide concentration of 63.64 µg/ml and 79.92 µg/ml were produced in peanut and cowpea media respectively. The least concentration of peptides of 25.83 µg/ml and 37.00 µg/ml in peanut and cowpea media were produced at 72 h of fermentation. *L. plantarum* displayed a significant increase in protease activity (quantitative) at 24 h of fermentation followed by a subsequent decrease with both parent proteins. The same medium which encouraged higher count had the highest protease activity of 125.88 ± 0.72 U/ml at 24 h. Similar finding was reported by Vij *et al*. (2014), who observed higher proteolytic activity of 839 tyrosine units / ml with *Lactobacillus* spp. Proteolytic hydrolysis of *L. plantarum* could release encrypted amino groups of long or short sequence (peptide) which could be assessed through OPA method (Hati *et al*., 2015). The result from OPA assay using serine as standard amino group revealed that *L. plantarum* fermented cowpea displaying higher peptide concentration than peanut which could be as a result of its high growth rate in the medium. In addition, the highest protease produced at 24 h in cowpea medium could break down protein to peptides, hence resulting to peptide accumulation at 48 h of fermentation. The peptide concentration at 48 h is higher than the original peptide constituent of flour at 0 h prior to fermentation. This increase after fermentation is as a result of the proteolytic action of proteases produced by *Lactobacillus plantarum*. The protease breakdown protein into their lower molecular weight peptides and amino acid sequences. These encrypted amino sequences confer different bioactive activities (Singh *et al*., 2015). Aqueous crude peptide extracts obtained from cowpea and peanut media displayed different level of antibacterial activity against the test isolates used (Table 6). Crude extract from peanut had the highest antibacterial activity exhibited by an inhibition zone of 18.00 ± 0.20 against *E. coli* isolates. Cowpea derived extract displayed the least inhibition zone of 11.00 ± 0.00 against *E. coli*. This possibly suggests defatted peanut medium to be a preferable substrate for antimicrobial peptide production than cowpea. In a study conducted by Rana and Bajaj (2015) on the antimicrobial peptides generation from milk proteins by Lactobacillus spp. under different conditions, they discovered a negative correlation among proteolysis activity and antimicrobial activity. As a result of loss in active peptide formed and appearance of new ones (Donkor *et al*., 2007). Furthermore, uninoculated peanut broth which was used as the control reflected zone of inhibition of 8mm and 6mm against *E.coli* and *Salmonella enteritidis* respectively. This is possible because peanut seed (and other plant material) has been shown to contain secondary metabolites like phytochemical which confer antibacterial activity and other bioactive activities (Mythily and Revathi, 2017). Therefore, cowpea and peanut proteins are utilizable for *L. plantarum* growth, proteolytic activity and peptide release but the antibacterial activity of crude peptide extracts could be attributed to the combined effects of diverse peptide fractions with different amino acid composition (not solely serine amino group as reflected in this study).

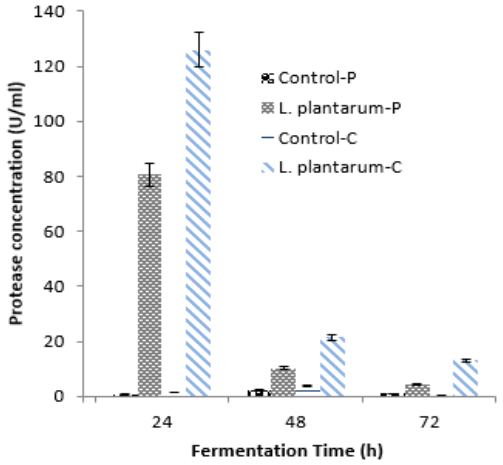

Fig 2: Protease activity of fermented dehulled defatted peanut and cowpea for a fermentation period of 72h. Key: P-peanut; C-cowpea.

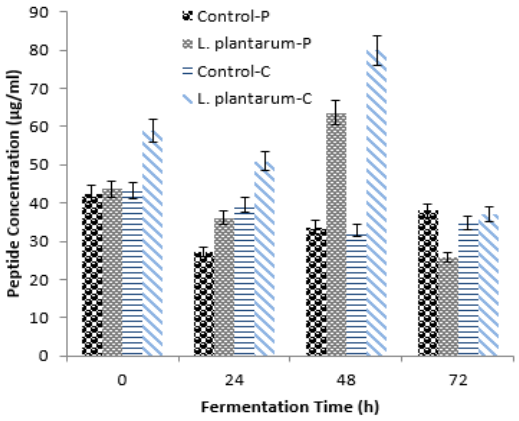

Fig 3: Peptide content in crude freeze dried-fermented peanut and cowpea. Values are mean ± standard error of triplicate. Key: P-peanut; C-cowpea.

Table 6: Antibacterial activity of crude products from freeze dried fermented dehulled defatted legume seeds against bacterial isolates

Test Isolates	Peanut		Cowpea		*P*-value
	Control	*L. plantarum*	Control	*L. plantarum*	
B. cereus	0.00 ± 0.00^{a}	8.00 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	<0.05
S. enteritidis	6.00 ± 0.30^{b}	17.00 ± 0.00^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00
E. cloacae	0.00 ± 0.00^{a}	13.00 ± 0.20^{b}	0.00 ± 0.00^{a}	12.00 ± 0.00^{c}	0.00
K. variicola	0.00 ± 0.00	16.00 ± 0.00	0.00 ± 0.00	16.00 ± 0.00	<0.05
P. aeruginosa	0.00 ± 0.00^{a}	12.00 ± 0.20^{b}	2.00 ± 0.00^{c}	14.00 ± 0.00^{d}	0.00
S. aureus	0.00 ± 0.00	12.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	<0.05
E. coli	8.00 ± 0.00^{a}	18.00 ± 0.20^{b}	4.00 ± 0.00^{c}	11.00 ± 0.00^{d}	0.00

Values are mean ± standard deviation of duplicate.

*Conclusion***:** This study has shown that *L. plantarum* CAU4347 could proteolyze peanut substrate best compared to cowpea for encrypted antibacterial peptides release from the parent protein. These antibacterial peptides are antagonistic against some meat spoilage isolates, hence their possible usage as meat bio-preservative.

REFERENCES

Abbasiliasi, S; Shun-Tan, J; Azmi, T; Ibrahim, T; Bashokouh, F; Ramakrishnan, N; Mustafaab, S; Ariff, A (2017). Fermentation Factors Influencing the Production of Bacteriocins by Lactic Acid Bacteria: A Review. The Royal Soc. Chem. Adv. 7: 29395–29420.

Abdel-Shafi, S; Mohammadi, A; Osman, A; Erian, G; Abdel-Hameid, S; Sitohy, M (2019). Characterization and antibacterial activity of 7S and 11S globulins isolated from cowpea seed protein. *Molecules* 24(6): 1082.

Adeyemo, SM; Onilude, AA (2013). Enzymatic reduction of anti-nutritional factors in fermenting soybeans by *Lactobacillus plantarum* isolates from fermenting cereals. *Nig. Food J.* 31(2): 84-90.

Agyei, D; Danquah, KM (2012). Rethinking food-derived bioactive peptides for antimicrobial and immunomodulatory activities. *Trends in Food Sci. Tech.* 23(2): 62-69

Aluko, ER (2015). Antihypertensive peptide in food proteins. *Ann. Rev. Food Sc. Tech.* 6: 235-262.

Alvarez-Ordonez, A; Begley, M; Clifford, T; Deasy, T; Consldine, K; Hill, C (2013). Structure-activity relationship of synthetic variants of the milk-derived antimicrobial peptide αs2-casein f (183-207). *App. Environ. Micro.* 79(17): 5179-5185.

Association of Official Analytical Chemistry (AOAC) (1990). *Official Methods of Analysis (15th Ed)*.Washington, D C, 368pp.

Balakrishnan, B; Prasad, B; Rai, KA; Suresh, PV; Subbanna, NM; Narayan, B (2011). *Invitro* antioxidant and antibacterial properties of hydrolyzed proteins of delimed tannery fleshings: comparison of acid hydrolysis and fermentation methods. *Biodeg.* 22(2): 287-285.

Biobaku, KT; Thomas, A; Aremu, NT; Asogwa, SA; Akorede, GJ; Basiru, A (2017). Nutritive effects of fermented *Parkia biglobosa* seeds on recovery of malnourished rats. *Ceylon J. Sci.* 46(3): 47-53.

Chalamaiah, M; Kumar, DB; Hemalatha, R; Tummala, J (2012). Fish protein hydrolyzates: proximate, composition, amino acid composition, antioxidant activities and applications: A review. *Food Chem*. 135(4): 3020-3038.

Cheesbrough, M (2000). *Distinct Laboratory Practice in Tropical Countries*, Part 2. (2nd Ed). Cambridge University Press, Cambridge 442 pp.

Da Trindade, CL; Marques, E; Lopez, BD; Ferraira, VSAM (2007). Development of a molecular method of detection and identification of *Xanthomonas campestris* pv. Viticola. *Summa Phytopathologica* 33(1):16-23.

Demirci, S; Ustaoglu, Z; Yilmazer, AG; Sahin, F; Bac, N (2014). Antimicrobial properties of zeolites-X and zeolite-A ion exchanged with silver, copper and zinc against abroad range of microorganisms. *Appl. Biochem. Biotech.* 172: 1652-1662.

Dhayakaran, R; Neethirajan, S; Weng, X (2016). Investigation of the antibacterial activity of soy peptides by developing a high throughput drug screening assay. *Biochem. Biophy. Reports* 6: 149-157.

Donkor, O; Henriksson, A; Vasiljevic, T; Shah, NP (2007). Proteolytic activity of diary lactic acid bacteria and probiotics as determinant of growth and *invitro* angiotensin-converting enzyme inhibitory activity in fermented milk. *Le Lait 87*: 21-28.

Godbey, TW (2014). Fermentation, beer and biofuel. In: An Introduction to Biotechnology: the Science, Technology and Medical Application Woodhead Publishing, Cambridge, United Kingdom, p331-351.

Hartmann, R. and Meisel, H (2007). Food-derived peptides with biological activity: from research to food applications. *Food Biotech* 18:163-169.

Hati, S; Sreeja, V; Solanki, J; Prajapat, JB (2015). Significance of proteolytic microorganisms on ACE-inhibitory activity and release of bioactive peptides during fermentation of milk. *J. Dairy Sci.* 68(6): 584-597.

Holzapfel, WH (2002). Appropriate starter-culture technologies for small-scale fermentation in developing countries. *Int'l. J. Food Microb.* 75: 197-212.

Jakubczyk, A; Karas, M; Baraniak, B; Pietrzak, M (2013). The impact of fermentation and in vitro digestion on formation angiotensin converting enzyme (ACE) inhibitory peptides from pea proteins. *Food Chem.* 141:3774–80.

Khalid, II; EIharadallou, SB (2013). Functional properties of Cowpea (*Vigna Ungiculata* L.Walp.), Lupin (*Lupinus termis*) flour and protein isolates. *J. Nutr. Food Sci.* 3(6): 2-6.

Korhonen, H; Pihlanto, A (2006). Bioactive peptides: production and functionality. *Int'l. Dairy J.* 16: 945-960.

Lapsongphon, N; Yongsawatdigul, J (2013). Production and purification of antioxidant peptides from a mungbean meal hydrolyzate by *Virgibacillus* sp. SK37 proteinase. *Food Chem.* 141: 992-999.

Liu, B; Chang, P (2008). Production of hydrolysate with antioxidative activity and functional properties by enzymatic hydrolysis of defatted sesame (*Sesamum indicum* L.). *Int'l. J. App. Sci. Eng.* 6(2): 73-83.

Mohanty, DP; Mohapatra, S; Misra, S; Sahu, PS (2016). Milk derived bioactive peptides and their impact on human health-A review. *Saudi J. Bio. Sci.* 23: 577-583.

Mythily, K; Revathi, K (2017). Preliminary phytochemical analysis and antimicrobial evaluation of *Arachis hypogea* L. *Int'l. J. Curr. Res. Med. Sci.* 3(3): 76-82.

Nwafor, IF; Egonu, NS; Nweze, ON; Ohabuenyi, NS (2017). Effects of processing methods on the nutritional values and anti-nutritive factors of *Adenanthera pavonina* L. (Fabaceae) seeds. *Afri. J. Biotech.* 16(3): 106-112.

Ogbeibu, AE (2005). *Biostatistics: A Practical Approach to Research and Data Handling*. Mindex Publishing Company Limited, Benin City Edo State, Nigeria 264pp.

Osman, AO; Mahgoub, SA; Sitohy, MZ (2013). Preservative action of 11S (glycinin) and 7S (β-conglycinin) soy globulin on bovine raw milk stored either at 4 or 25 C. *J. Dairy Res.* 80:174–183.

Pailin, T; Kang, DH; Schmidt, K; Fung, DYC (2001). Detection of extracellular bound proteinase in EPS-producing lactic acid bacteria cultures on skim milk agar. *J Appl. Microb.* 33(1):45-49.

Park, M; General, T; Lee S (2012). Physicochemical properties of roasted soybean flour bioconverted by solid-state fermentation using *Bacillus subtilis* and *Lactobacillus plantarum. Prev. Nutri. Food Sci.* 17: 36-45.

Phulia, V; Sardar, P; Sahu, PN; Sanap, NB (2018). Effect of detoxification methods on anti-nutritional factors and proximate composition of defatted *Jatropha curcas* kernel meal. *Animal Nutrition and Feed Technology* 18(1): 67-77.

Rana, S; Bajaj, R (2015). Optimization of conditions for generation of antimicrobial peptides from milk proteins by *Lactobacillus* sp. *Afri. J. Microb. Res.* 9(24): 1573-1585.

Rawat, S (2015). Food spoilage: microorganisms and their prevention. *Asian J. of Pt. Sci. Res.* 5(4): 47-56.

Singh, BP; Vij, S; Hati, S; Singh, D; Kumari, P; Minj, J (2015). Antimicrobial activity of bioactive peptides derived from fermentation of soymilk by *Lactobacillus plantarum* C_2 against foodborne pathogens. *Int'l. J. Ferm. Foods* 4: 91-99.

Slivinski, CT; Mallmann, E; De Araujo, MJ; Mitchell, AD; Krieger, N (2012). Production of surfactin by *Bacillus pumilus* UFPEDA 448 in solid state fermentation using a medium based on okara with

sugar cane bagasse as a bulking agent. *Process Biochem.* 47: 1848-1855.

Thu, TV; Foo, HL; Loh, TC; Bejo, MH (2011). Inhibitory activity and organic acid concentration of metabolite combinations produced by various strains of *L. plantarum*. *Afr. J. Biotech.* 10: 1359-1356.

Toe, JC; Foo, LH; Loh, CT; Mohammad, R; Rahun, AR; Idrus, Z (2019). Extracellular proteolytic activity and amino acid production by lactic acid bacteria isolated from Malaysian foods. *Int'l. J. Mol. Sci.* 20: 1777-1799.

Udenigwe, CC; Aluko, ER (2012). Food protein-derived bioactive peptides: production, processing and potential health benefits. *J. Food Sci.* 1: 327-341.

Vij, S; Chandra, P; Bachanti, RP (2014). Antimicrobial activity of casein fermentate of probiotic *Lactobacillus* spp. *Int'l J. Genetic Engineer. Biotech.* 5(2): 201-208.

Virtanum, T; Pihlanto, A; Akkanen, S; Korhonen, H (2007). Development of antioxidant activity in milk whey during fermentation with lactic acid bacteria. J. Appl. Micro. 102(1): 106-115.

Wang, YK; He, HL; Chen, XL; Sun, CY; Zhang, YZ; Zhou, BC (2008). Production of novel angiotensin I converting enzyme inhibitory peptides by fermentation of marine shrimp (*Acetes chinensis*) with *Lactobacillus fermentum* SM 605. *Appl. Microb. Biotech.* 79(5): 785-791.

Wu, H; Wang, Q; Ma, T; Ren, J (2009). Comparative studies on the functional properties of various protein concentrate preparations of peanut protein. *Food Res. Int'l.* 42(3): 343-348.