AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Improving microbial growth and biological production levels of fermented palm kernel cake based on solid state fermentation using *Bacillus velezensis* with supplementation of reducing sugar from molasses and nitrogen from urea

Patsaporn Pongmalai[a,*], Apichaya Sae-Teng[a], Saisunee rattanakaruna[a], Thananon Yuenyang[a], Sunun Siriraksophon[a,b]

[a] *Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi (Bang Khun Thian), Bangkok 10150, Thailand*

[b] *Biochemical Engineering and Systems Biology Research Group, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency,King Mongkut's University of Technology Thonburi (Bang Khun Thian), Bangkok 10150, Thailand*

Article Info

Article history:
Received 20 August 2024
Revised 20 February 2025
Accepted 10 March 2025
Available online 28 April 2025

Keywords:
Amylase activity,
Palm kernel cake (PKC),
Protease activity,
Protein content,
Total viable count (TVC)

Abstract

Importance of the work: Palm kernel cake (PKC), a by-product from the palm oil industry containing 14%–18% protein, is well-known as an alternative protein ingredient to reduce the cost of animal feed. However, the utilization of cellulosic PKC as an animal feed ingredient is still limited. Improving the nutritional value and protein content of PKC based on solid state fermentation (SSF) with useful probiotics would expand the utilization of PKC as an ingredient in poultry or monogastric animals.

Objectives: To investigate the effects of SSF of PKC using *Bacillus velezensis*, together with adding supplements, to help increase microbial growth and biological production levels.

Materials and Methods: Suitable conditions were investigated for culturing *B. velezensis* based on SSF using PKC as a substrate with the addition of reducing sugar (RS) from molasses and nitrogen (N) from urea as microbial supplements. The total viable count (TVC), enzyme activity levels and protein content of the fermented PKC were considered for the conditions selected.

Results: Fermentation of PKC with the addition of 20 g RS/kg sample and without N addition for 5 d produced high values for TVC (10.05 log CFU/g sample, 1.16×10^{10} CFU/g sample), soluble protein content (17.51 mg/g sample on a dry basis, d.b.) and high levels of protease and amylase activity levels (49.41 international units (U)/g sample (d.b.) and 739.13 U/g sample (d.b.), respectively). The crude protein content based on proximate analysis of the fermented samples increased from 13.79% to 20.98%, which was 1.52-fold higher than for only PKC, while the crude fiber of the fermented PKC was slightly reduced from 27.11% to 25.40% (a 0.94-fold decrease).

Main finding: Processing of PKC based on SSF using *B. velezensis* with the addition of RS at 20 g RS/kg sample for 5 d was a suitable biological production process to improve the microbial growth and nutritional value of fermented PKC and would be applicable for probiotic production.

* Corresponding author.
E-mail address: patsaporn.pon@kmutt.ac.th (P. Pongmalai)

https://doi.org/10.34044/j.anres.2025.59.2.06

Introduction

The production of palm oil in Thailand has received considerable attention each year. Palm kernel cake (PKC) is one of the main by-products of the palm oil industry and involves agricultural industrial waste (Bello et al., 2018). PKC contains high amounts of crude protein (14–18%), fat (3–9%) and crude fiber (12–20%) (Sathitkowitchai et al., 2018; Azizi et al., 2021). Consequently, it has been applied as an alternative animal feed ingredient to reduce costs, replace insufficient protein in animal feed and to add product value to agricultural waste (Azizi et al., 2021). The crude fiber of PKC is acceptable to most ruminants; however, it may not be suitable if included at high levels in poultry diets, with the recommended inclusion level of PKC in broiler diets being up to 20–30%, which did not affect their growth performance or feed efficiency (Egenuka et al., 2018). However, an increase in the percentage of PKC utilization in poultry is still of interest for low-cost production.

Solid state fermentation (SSF) is an attractive means to process animal feed due to its low production cost and high product yield (Lomthong et al., 2021). It has been used in a wide range of applications to improve the biomass and nutritional values of by-products. Most *Bacillus* probiotics are used in SSF to improve the nutritional value of the products. For example, Zeng et al. (2021) reported that *Bacillus velezensis* K8 efficiently degraded lignocellulose in both brewer's spent grain and ultrasonic-pretreated brewer's spent grain, enhancing the soluble sugar and protein contents using SSF. In addition, *B. velezensis* is a probiotic used as an additive to improve health for use in animal feed (Khalid et al., 2021). Cui et al. (2024) studied the effect of *Bacillus velezensis* as supplementation on *Campylobacter jejuni* colonization in chickens. They reported that the growth of *C. jejuni* decreased from 7.46 $\log_{10}$ colony forming units (CFU)/mL to 1.02 $\log_{10}$ CFU/mL when *B. velezensis* was applied for 48 hr. In addition, Zhu La et al. (2024) reported that dietary supplementation with *Bacillus velezensis* CML532 promoted chicken growth and improved digestive and absorptive functions, intestinal morphology and barrier function. Products obtained using SSF, such as digestive enzymes (including protease, amylase and xylanase), can be utilized for animals. Pranay et al. (2019) investigated amylase production from agrowastes using SSF with *Bacillus subtilis* KR1. Wheat bran yielded the highest amylase production (82.60 international units (units per gram of substrate, U/gds), while rice bran produced 80.51 U/gds. Similarly, Šelo et al. (2021) demonstrated that SSF of wheat straw using *Bacillus* sp. BBXS-2 resulted in protease and amylase activity levels of 12,200 U/g dry matter and 6,900 U/g dry matter, respectively.

Protease and amylase are well-known as effective enzymes that can enhance intestinal digestibility and promote the growth performance of animals (Yin et al., 2018). Protease is a class of enzyme that catalyzes the reaction by breaking down proteins through the hydrolysis of peptide bonds (Morohashi and Tomita, 2013). Proteases can be produced by animals, plants and microbes that have potential applications in industries such as food processing, pharmaceuticals, and animal feed (Ojo-Omoniyi et al., 2024). Supplementation of animal feed with exogenous protease improves protein digestibility. For example, Oxenboll et al. (2011) demonstrated that the addition of protease in poultry feed enhanced the protein digestibility of broilers and improved nitrogen utilization. Qiu et al. (2023) studied the effects of protease concentrations on the amino acid digestibility of broilers. They reported that dietary supplementation with 250 mg/kg of protease increased the apparent ileal digestibility of crude protein and amino acids, while only cysteine, aspartic acid and glycine increased when 500 mg/kg of protease was applied. Furthermore, they noted that the addition of protease led to protein hydrolysis, inducing the absorption of intact protein and improving the growth performance of the broilers. Amylase is a digestive enzyme that breaks down starch into sugar molecules and is normally used in animal feeds to enhance nutrient digestion and promote growth performance (Stefanello et al., 2017). Gracia et al. (2003) mentioned that the addition of exogenous α-amylase in broiler feed (corn-soybean meal diet) could improve daily gains by 9.4% on day 7. The daily gain of broilers on days 0–21 and days 21–42 increased by 3.6% and 5.5%, respectively. Consequently, supplementation of α-amylase in a corn-soybean meal diet enhanced the digestibility of nutrients and the growth performance of broilers. Kaczmarek et al. (2014) reported that adding protease and amylase in broiler chicken feeds (corn-soybean meal-based diets) improved broiler chickens' body weight gain and feed conversion ratio during the first 2 wk of growth. Additionally, preliminary study to the present research on the enzyme-producing ability of *B. velezensis* before SSF showed that enzymes produced by *Bacillus velezensis* (protease and amylase) had the highest hydrolytic enzyme activity levels of 1,067.02 U/g dry weight of biomass and 14,834.98 U/g dry weight of biomass after culturing in selective media for 96 hr.

Thus, it seems useful to enhance microbial growth and biological production by adding inexpensive supplements to fermentation. Molasses, a low-value by-product from the sugar industry, has been applied widely in animal feed for many years (Sjölin et al., 2022). The major components of molasses are 31.05% (weight per weight, w/w) reducing sugar (RS), 9.49% (w/w) glucose, 1.16% (w/w) galactose, 9.95% (w/w) fructose and 2.90% (w/w) sucrose. Furthermore, molasses contains 2.5–4.5% crude protein, vitamins and minerals such as iron, zinc and copper (Heidari et al., 2011). Besides its nutritional benefits and availability, molasses-rich reducing sugar (RS) has been used to promote microbial growth in various applications (Vinale et al., 2020). Additionally, nitrogen (N) is recommended as a crucial source for microbial growth. Almost all microorganisms can grow better in organic nitrogen (present in some amino acids, such as peptone and tryptone, and urea) than in inorganic sources (Berovic and Podgornik, 2016). Urea contains 46% N and usually is utilized in feed as a protein supplement because it is cheap and non-toxic to animals (Lunsin et al., 2018). Nitrogen from urea can be applied as a nitrogen source for microorganisms to use as a precursor of protein synthesis and a source of energy, as well as for a nutritional additive, providing a source of non-protein nitrogen for ruminants (Niazifar et al., 2024). Wang (2012) reported that the addition of 1% (w/w) urea to soluble distiller's dried grains undergoing SSF using *Trichoderma reesei* led to the highest xylanase activity (51.7 U/g) compared to other treatments. Recently, Wang et al. (2023), who studied the use of different carbon sources to improve the nutritional values of corn straw and rice straw via SSF using *Phanerochaete chrysosporium*, suggested that molasses and glucose were the most effective carbon sources to improve the crude protein contents as well as reducing the lignin in the corn straw and rice straw after fermentation for 14 d. Nevertheless, limited information is available regarding adding RS from molasses and N from urea at different concentrations into fermented PKC during SSF.

Therefore, the present work aimed to investigate suitable conditions for applying SSF to PKC using *Bacillus velezensis*, which is a probiotic bacterium, together with adding molasses and urea as supplements to improve microbial growth (measured as the total viable count, TVC), enzyme activity and the protein content.

Material and methods

Material, microorganism and chemicals

Palm kernel cake (PKC) containing 14% crude protein at a moisture content of around 7% (wet basis, w.b.) was obtained using an expeller-pressed processor (Kim Kee Kicharoen Co., Ltd.; Thailand).

Bacillus velezensis was reidentified from *Bacillus licheniformis* TISTR 013 using 16S rRNA gene sequence analysis, which is commonly used for bacterial identification (Jenkins et al., 2012). This bacterium was received from the Thailand Institute of Scientific and Technological Research, Thailand.

Bovine serum albumin was obtained from Capricorn Scientific (Germany), while D-glucose was purchased from Ajax Finechem (New Zealand). L-tyrosine was obtained from Sigma-Aldrich (USA).

Preparation of inoculum

B. velezensis was cultured in a yeast extract peptone dextrose (YPD) broth containing 0.5 g yeast extract, 1 g peptone and 1 g D-glucose for a total volume of 50 mL. The inoculum was incubated at 37°C and shaken at 200 revolutions per minute (rpm) in an incubator shaker for 24 hr. The optical density at 600 nm (OD_{600}) of around 1.0 was selected as the final inoculum dilution.

Preparation of palm kernel cake sample

First, the PKC sample was prepared by mixing PKC with 100 mM phosphate buffer to reach a final pH and moisture content of the mixing sample of 6.5–7.0% and 50–65% (w.b.), respectively. Then the prepared PKC was divided into the designated experiments: 1) negative control sample (non-fermented sample without RS and N additions); 2) fermented PKC without RS and N additions; and 3) fermented PKC with RS and N additions.

The samples of the prepared fermented PKC with RS and N additions were allocated to added molasses containing RS at 2.5 g RS/kg sample, 5 g RS/kg sample, 10 g RS/kg sample, 15 g RS/kg sample and 20 g RS/kg sample. Notably, the RS was derived from molasses, which was obtained from Rajburi Sugar Co., Ltd., Thailand and had 44% (weight per weight, w/w) RS. The N was derived from urea and was fixed at 1 g N/kg sample

and also added into all fermented samples. Next, 30 g of each mixed sample was placed in a 250 mL Erlenmeyer flask, which was covered with cotton and aluminum foil. Then, all flasks were sterilized at 121°C for 15 min and subsequently cooled to room temperature prior to SSF. The suitable RS concentration was selected for further study based on the addition of N from urea at 5 N/kg sample, 10 N/kg sample, 15 N/kg sample, 20 N/kg sample or 25 g N/kg sample.

Solid state fermentation

The 10% (v/w) inoculum of *B. velezensis* was mixed with 30 g each of the sterilized PKC samples containing RS and N additions (but not with the negative control sample). All samples were fermented at 37°C for 9 d. Subsamples were collected at days 0, 1, 3, 5, 7 and 9 for analysis.

Determination of total viable count

A sample (1 g) of fermented PKC was mixed with 9 mL of sterilized water; with sufficient diluted suspension to make at least three different dilutions. Then, 100 µL of the suspension was spread on a YPD agar plate. A sterile stainless-steel spreader was used to distribute a microbial population of bacteria on the plate. Then, the plate was covered and placed in a sealed plastic bag prior to incubation at 37°C for 24 hr. A countable range of colonies was defined as 25–250 colonies/plate. Colony counts were expressed as colony forming units per gram of sample (log CFU/g sample).

Determination of moisture content

A sample (5 g) of PKC was placed in an aluminum can; the samples were spread uniformly prior to drying. Moisture content analysis was conducted by drying at 105°C for 24 hr, following Association of Official Analytical Chemists (2012).

pH measurement

Each PKC sample (1 g) was mixed with 10 mL of distilled water. Then, the mixture was measured for pH using a pH meter.

Determination of reducing sugar content

The RS content of each sample was determined using the dinitrosalicylic acid (DNS) method following Saqib and Whitney (2011), with some modifications. Each PKC sample (5 g) was mixed with 50 mL of reverse osmosis (RO) water and the mixture solution was stirred at 200 rpm for 15 min. About 250 µL of the mixture was placed in a test tube. Then, 1 mL of DNS reagent was added into the tube. The tube was covered and placed in boiling water for 5 min and subsequently rapidly cooled to room temperature. The absorbance was measured at 540 nm using an ultraviolet-visible light spectrophotometer (Metertech; SP-830 PLUS; Taiwan).

Protease activity assay

The protease activity was determined using a modified method from Boratyñski et al. (2018). Each PKC sample (5 g) was mixed with 50 mL of 20 mM phosphate buffer (pH 7.2). The mixture solution was stirred at 200 rpm for 15 min prior to centrifugation at 9,000 ×*g* for 5 min. Next, 0.5 mL of supernatant was mixed with 1 mL of 0.6% (weight per volume, w/v) casein in 0.1 M Tris-HCl (pH 8.0) at 37°C for 10 min; Tris buffer at pH 8.0 and 37 ºC was used as the reported most effective condition for protease activity assay (Banik et al., 2018). About 2.5 mL of 0.11 M trichloroacetic acid was added to stop the reaction. Then, 1 mL of the mixture was mixed with 5 mL of 0.4 M sodium carbonate and 1 mL of 3× dilution of Folin-Ciocalteu reagent, respectively. The reaction mixture was carried out at 37°C for 20 min; the absorbance of the mixture was measured at 660 nm. Tyrosine concentrations (0–500 ppm) were used to develop a standard curve. One unit of enzyme was defined as an amount of the enzyme required to liberate 1 µmol of tyrosine per min. Protease activity was expressed as a unit (U)/g sample (d.b.).

Amylase activity assay

The amylase activity was determined following the method of Afrisham et al. (2016), with some modifications. Each PKC sample (5 g) was mixed with 50 mL of 100 mM phosphate buffer (pH 7.0). The mixture solution was stirred at 200 rpm for 15 min and then centrifuged at 9,000 ×*g* for 5 min to separate particles from the solution. About 0.5 mL of supernatant phosphate buffer (pH 7.0) was mixed with 0.5 mL 1% (w/v) soluble starch solution. The optimum buffer and pH for amylase activity assay were reported in the pH range 4.5–7.0 (Yadav and Prakash, 2011). The reaction was carried out for 15 min at 50°C, which has been reported to be the optimum

temperature for amylase activity (Bano et al., 2011). Then 1 mL of DNS reagent, prepared following Saqib and Whitney (2011) with some modifications, was added to the mixture and boiled for 5 min prior to cooling to room temperature. The reaction mixture was measured at 540 nm and D-glucose at concentrations of 0–1,000 ppm was used to develop a standard curve. One unit of enzyme was defined as the amount of the enzyme required to liberate 1 µmol of glucose per min. Amylase activity was expressed as a U/g sample (d.b.).

Determination of soluble protein content

The soluble protein content in each PKC sample was determined following Bradford (1976), with some modifications. Each PKC sample (5 g) was mixed with 50 ml of RO water and stirred at 200 rpm for 15 min. Then, the mixture was centrifuged at 9,000 ×*g* for 5 min. Subsequently, 10 µL of supernatant was mixed with 200 µL of Bradford dye reagent. The mixture was left at room temperature for 15 min and then measured at 595 nm. The protein content was calculated from a standard curve of bovine serum albumin based on concentrations in the range 0–1,000 µg/L.

Determination of proximate chemical compositions

Standard methods were applied to determine the proximate chemical components (crude protein, crude fiber, ash and fat contents). Specifically, the crude protein and crude fiber contents of each sample were determined according to the standard method of Association of Official Analytical Chemists (2012). The ash content was determined according to the standard method of American Society for Testing and Materials D3174-12 (2012), while the fat content was analyzed based on the standard method of American Public Health Association (2017).

Statistical analysis

Experimental data were presented as mean ± SD values after being subjected to analysis of variance analysis. Duncan's new multiple range test was used to determine differences in mean values, which were considered at the 95% confidence level for significance ($p < 0.05$) using the SPSS® software version 17 (SPSS Inc.; USA) for statistical analysis. All experiments were independently carried out at least in duplicate.

Results and discussion

Initially, RS at different concentrations was added to the PKC samples that had undergone SSF using *B. velezensis* to determine a suitable RS concentration that would enhance the TVC, enzyme activity and the protein content. The selected RS concentration was later applied to determine the suitable fermentation process of PKC by varying N at different concentrations.

Addition of reducing sugar at different concentrations

RS from molasses at different concentrations was added, while N from urea was fixed at 1 g N/kg sample. Both the RS and N were mixed with PKC before fermentation for 9 d.

Fig. 1A shows the TVC values of *B. velezensis* on fermented PKC that had undergone RS additions of 0 g RS/kg sample, 2.5 g RS/kg sample, 5 g RS/kg sample, 10 g RS/kg sample, 15 g RS/kg sample or 20 g RS/kg sample with 1 g N/kg sample compared to the negative control sample. At day 0, the initial TVC of all fermented samples was in the range 6.11–6.35 log CFU/g sample (or 1.30×10^6–2.24×10^6 CFU/g sample). The TVC values increased continuously until reaching their maxima of 10.05 log CFU/g sample, 9.99 log CFU/g sample and 10.07 log CFU/g sample (or 1.13×10^{10} CFU/g sample, 1.24×10^{10} CFU/g sample and 1.17×10^{10} CFU/g sample) for the fermented samples with 5 RS/kg sample, 10 RS/kg sample and 15 g RS/kg sample, respectively, at day 5 and of 10.07 log CFU/g sample and 10.14 log CFU/g sample (or 1.18×10^{10} CFU/g sample and 1.37×10^{10} CFU/g sample) for the fermented samples with 2.5 RS/kg and 20 g RS/kg at days 9 and 7, respectively. The maximum TVC for the fermented samples without RS addition (0 g RS/kg) was 9.89 log CFU/g (or 7.75×10^9 CFU/g sample) on day 5. There was no significant difference in the microbial population compared to the other fermented samples, suggesting that the RS nutritional value of PKC as a substrate might be adequate for bacterial growth during fermentation. After that, the TVC of almost all the fermented samples remained unchanged until the end of fermentation. Notably, there was no noticeable growth of bacterial colonies in the negative control samples during fermentation. Therefore, *B. velezensis* could grow on the fermented PKC with and without RS addition.

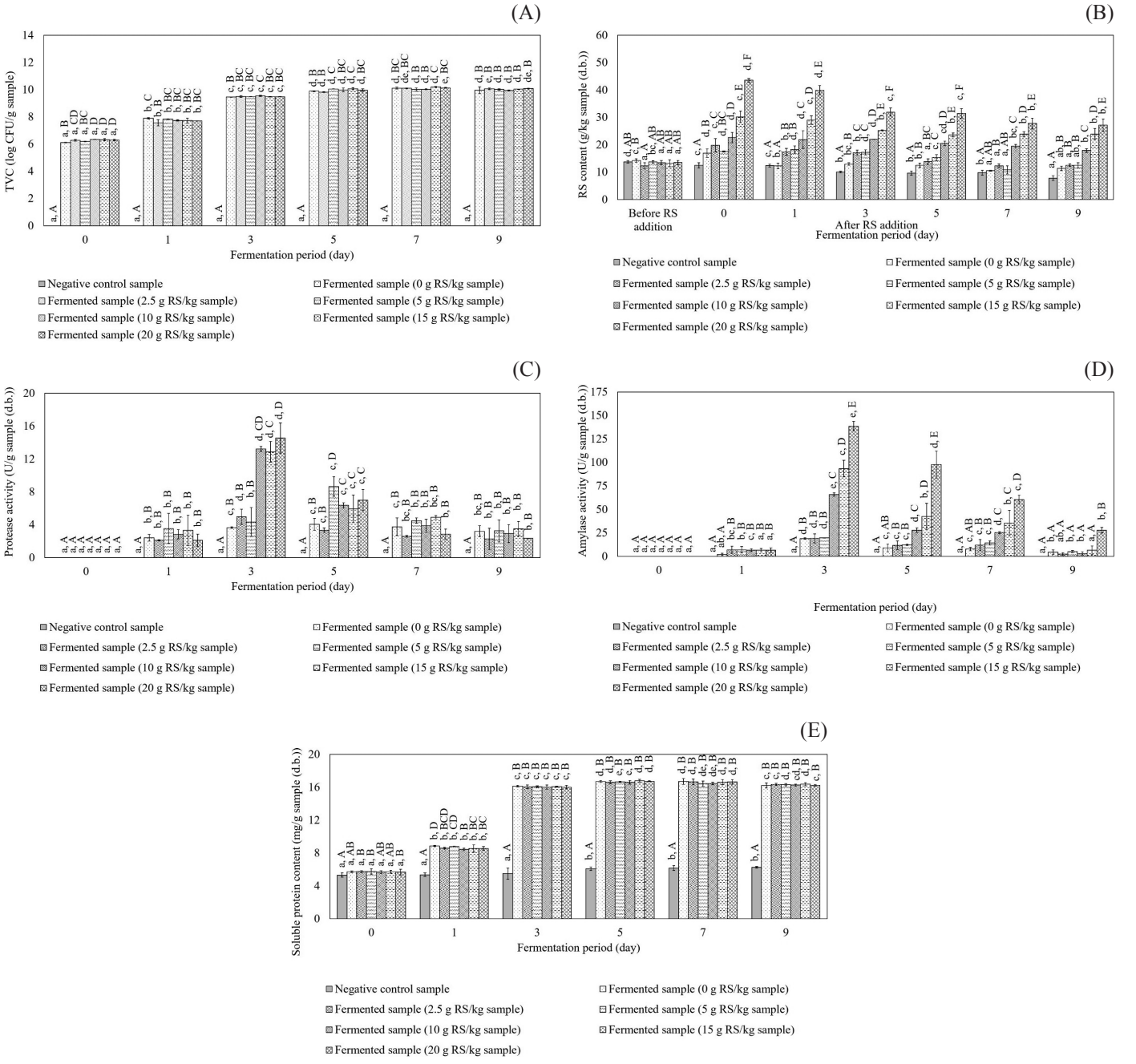

Fig. 1 Changes in total viable count (TVC), reducing sugar (RS) contents, enzyme activity levels and soluble protein contents of palm kernel cake with added 1 g N/kg sample in different RS amounts undergoing solid state fermentation at 37°C for 9 d: (A) TVC; (B) RS content; (C) protease activity; (D) amylase activity; (E) soluble protein content. Bars with the same lowercase letters within the same sample and the same uppercase letters within the same fermentation time are not significantly ($p \geq 0.05$) different.

Generally, an increase in the TVC of bacteria depended on several parameters, including moisture content and pH during SSF. For example, the moisture contents of fermented samples that had undergone RS addition increased slightly during fermentation due to cellular respiration in microorganisms that affect water release (Epstein, 2017), while the negative control sample showed no change in moisture content (Table 1). The pH values of all the fermented samples clearly increased on day 3 and reached their maxima during days 3 and 5, with values in the range 7.41–7.76 (Table 2).

Table 1 Changes in percentage moisture content of palm kernel cake with different additions of reducing sugar (RS) and of N at 1 g N/kg sample undergoing solid state fermentation at 37°C for 9 d

Sample	Day 0	Day 1	Day 3	Day 5	Day7	Day 9
Negative control sample	57.24±0.31$^{a, A}$	57.35±0.70$^{a, A}$	57.43±0.35$^{a, A}$	56.02±2.10$^{a, A}$	56.59±2.16$^{a, A}$	54.92±0.85$^{a, A}$
Fermented sample (0 g RS/kg sample)	60.63±0.20$^{a, C}$	60.74±0.45$^{a, B}$	61.96±0.47$^{ab, B}$	62.14±0.82$^{b, B}$	61.67±0.58$^{ab, B}$	61.35±1.26$^{ab, BC}$
Fermented sample (2.5 g RS/kg sample)	61.00±0.65$^{a, C}$	61.76±0.23$^{ab, BC}$	63.54±0.60$^{c, B}$	62.38±0.32$^{b, B}$	61.67±0.60$^{ab, B}$	61.49±0.47$^{ab, BC}$
Fermented sample (5 g RS/kg sample)	60.82±0.26$^{a, C}$	61.57±0.13$^{a, BC}$	62.43±1.47$^{a, B}$	61.99±0.34$^{a, B}$	60.02±2.62$^{a, B}$	62.00±0.12$^{a, BC}$
Fermented sample (10 g RS/kg sample)	61.06±0.34$^{a, C}$	62.24±0.22$^{ab, C}$	62.81±1.02$^{b, B}$	62.38±0.34 $^{ab, B}$	62.22±0.65$^{ab, B}$	61.47±1.13$^{ab, BC}$
Fermented sample (15 g RS/kg sample)	60.58±0.72$^{a, C}$	61.01±1.52$^{a, BC}$	62.23±1.64$^{a, B}$	61.53±0.23$^{a, B}$	60.28±1.19$^{a, B}$	62.38±0.84$^{a, C}$
Fermented sample (20 g RS/kg sample)	60.02±0.91$^{a, B}$	60.91±0.34$^{ab, B}$	62.55±0.47$^{c, B}$	61.92±0.40$^{bc, B}$	61.16±1.09$^{ab, B}$	60.64±0.35$^{a, B}$

Values (mean ± SD) with the same lowercase superscript letters within the same sample and the same uppercase superscript letters within the same fermentation period are not significantly ($p \geq 0.05$) different.

Table 2 Changes in pH of palm kernel cake with different additions of reducing sugar (RS) and of N at 1 g N/kg sample undergoing solid state fermentation at 37°C for 9 d

Sample	Day 0	Day 1	Day 3	Day 5	Day7	Day 9
Negative control sample	6.64±0.04$^{d, D}$	6.46±0.06$^{c, D}$	6.37±0.05$^{b, A}$	6.24±0.03$^{a, A}$	6.26±0.03$^{a, A}$	6.21±0.03$^{a, A}$
Fermented sample (0 g RS/kg sample)	6.71±0.01$^{b, D}$	6.46±0.06$^{a, D}$	7.66±0.03$^{d, D}$	7.74±0.05$^{d, D}$	7.52±0.02$^{c, D}$	7.51±0.07$^{c, E}$
Fermented sample (2.5 g RS/kg sample)	6.53±0.05$^{b, C}$	6.34±0.03$^{a, BC}$	7.66±0.05$^{d, D}$	7.76±0.05$^{e, D}$	7.53±0.01$^{c, D}$	7.51±0.02$^{c, E}$
Fermented sample (5 g RS/kg sample)	6.44±0.13$^{a, C}$	6.38±0.01$^{a, CD}$	7.65±0.03$^{c, D}$	7.75±0.02$^{d, D}$	7.54±0.01$^{b, D}$	7.54±0.01$^{b, E}$
Fermented sample (10 g RS/kg sample)	6.34±0.01$^{b, B}$	6.27±0.03$^{a, B}$	7.52±0.01$^{d, C}$	7.66±0.03$^{e, C}$	7.45±0.07$^{c, C}$	7.40±0.02$^{c, D}$
Fermented sample (15 g RS/kg sample)	6.27±0.01$^{a, B}$	6.30±0.06$^{a, B}$	7.49±0.02$^{d, C}$	7.65±0.01$^{e, C}$	7.39±0.02$^{c, B}$	7.31±0.05$^{b, C}$
Fermented sample (20 g RS/kg sample)	6.12±0.04$^{a, A}$	6.14±0.08$^{a, A}$	7.41±0.04$^{c, B}$	7.51±0.04$^{c, B}$	7.34±0.03$^{c, B}$	7.21±0.05$^{b, B}$

Values (mean ± SD) with the same lowercase superscript letters within the same sample and the same uppercase superscript letters within the same fermentation period are not significantly ($p \geq 0.05$) different.

This was probably because the microorganisms had consumed organic acids that were produced during SSF, inducing increases in pH values (Williams, 2013). Notably, pH values of around 7 have been reported to be the optimum for the production of amylase (Nusrat and Rahman, 2007). Nevertheless, the pH of the samples in the present study decreased slightly during days 7–9.

The RS of the fermented and negative control samples is shown in Fig. 1B. Before RS addition, the contents of all samples were 12.28–14.24 g RS/kg sample. After adding RS, the RS of almost all fermented samples increased according to their concentrations. The RS of all the fermented samples had a declining trend toward the end of fermentation, since the RS had been utilized as a carbon source for cell proliferation (Jung, 2016). Decreases in the RS contents of all the fermented samples corresponded well with the increases in microbial populations, as presented in Fig. 1A.

The protease and amylase activity levels of the fermented samples, with and without RS addition, and of the negative control samples are illustrated in Fig. 1C and 1D (however, no measurements were carried out on day 0). The activity levels of these enzymes of the fermented samples, especially at 10 g RS/kg sample, 15 g RS/kg sample and 20 g RS/kg sample, increased and reached their maxima on day 3, with protease activity levels of 13.22 U/g sample (d.b.), 12.86 U/g sample (d.b.) and 14.56 U/g sample (d.b.) and amylase activity levels of 65.76 U/g sample (d.b.), 93.36 U/g sample (d.b.) and 138.63 U/g sample (d.b.), respectively. The protease and amylase activity levels of the other fermented samples also increased; however, their levels were lower than those obtained from the fermented samples mentioned previously. Higher RS concentrations (10 RS/kg sample, 15 RS/kg sample and 20 g RS/kg sample) induced higher enzyme activity levels, probably because of their increased utilization during cell growth and maintenance, improving enzyme production. Nevertheless, the enzyme activity levels of all the fermented samples decreased after day 3, perhaps due to the number of cells not increasing during the stationary phase because of nutrient exhaustion or the accumulation of enzyme repressors (Alrumman et al., 2018), leading to decreases in enzyme production. The fermented sample at 20 g RS/kg had the highest level for amylase activity and also had a high activity level for protease. Notably, the protease and amylase activity levels of the negative control sample were negligible until the end of the experiment.

Fig. 1E shows soluble protein contents of the fermented and the negative control samples. The contents of soluble protein in all the fermented samples clearly increased on day 3 and reached their maxima on day 5 in the range 16.60–16.77 mg/g sample (d.b.); these contents were not significantly different. The soluble protein contents of the fermented samples were related to their TVCs as the cell numbers increased, the soluble protein contents of all fermented samples increased. For the negative control sample, the soluble protein content of this sample changed only slightly during fermentation.

From the above-mentioned discussion, the fermented sample at 20 g RS/kg sample had the highest amylase activity compared to the other fermented samples, as well as high protease activity, bacterial populations and soluble protein content, similar to those of other conditions. Thus, this condition was selected for further study of N addition at different concentrations to optimize the fermentation process of the fermented PKC.

Addition of nitrogen at different concentrations

Based on the results in the previous section, 20 g RS/kg sample was selected as the suitable RS concentration of fermented PKC and applied in this section to investigate the optimum fermentation process.

There was an increase in the TVC of *B. velezensis* in the fermented samples with and without N additions (Fig. 2A). The bacterial viable counts of almost all the fermented samples increased continuously and reached their maxima during days 3 and 5; the TVC of these samples remained almost unchanged until the end of fermentation. On day 3, the fermented samples at 20 g RS/kg sample without N addition had a maximum viable count (9.64 log CFU/g sample (4.60×10^9 CFU/g sample)) similar to that of the fermented sample at 20 g RS/kg sample with 5 g N/kg, 10 g N/kg and 15 g N/kg, which had maxima of 10.18 log CFU/g sample, 10.00 log CFU/g sample and 9.79 log CFU/g sample, respectively, (or 1.54×10^{10} CFU/g sample 1.12×10^{10} CFU/g sample and 6.15×10^9 CFU/g sample, respectively). The fermented samples at 20 g RS/kg sample with 20 N/kg sample and 25 g N/kg sample, had lower TVCs than those of the other conditions. Thus, it seemed that high cell numbers were obtained when the fermented samples at 20 g RS/kg sample had low added N concentrations (0 g N/g sample, 5 g N/g sample, 10 g N/g sample and 15 g N/g sample). The cell metabolism of bacteria might have been disrupted by a high concentration of N, causing inadequate absorption of other essential nutrients and inducing the inhibition of cell growth (Chai and Adnan, 2018). Li et al. (2023) studied the effect of the carbon-to-nitrogen (C:N) ratio using sucrose as a carbon source and NH_4Cl as a nitrogen source on the product per substrate ($Y_{P/S}$) and product per biomass ($Y_{P/X}$) to improve *Bacillus subtilis* 168 growth. The optimum C:N ratio for $Y_{P/S}$ and $Y_{P/X}$ was 11.68:1, producing $Y_{P/S}$ and $Y_{P/X}$ values of 0.076 g/g and 0.46 g/g, respectively. However, a C:N ratio of 7:1 led to a decrease in $Y_{P/S}$ and $Y_{P/X}$ because the excess amount of N reacted with the C source, affecting the growth and metabolism of the bacteria. Nonetheless, the maximum TVCs of these fermented samples were similar to that obtained from the fermented sample at 20 g RS/kg sample with added 1 g N/kg sample (as presented in the previous section) in almost all cases and were in the range of probiotics (Marinova et al., 2019). According to the Ministry of Agriculture and Cooperatives in Thailand (2016), the quantity of microorganisms mixed into animal feed must be at least 1×10^5 CFU/kg of feed.

The moisture content and pH values (Tables 3 and 4) increased slightly in almost all cases. As presented in Fig. 2B, the decrease in RS was related to the increase in fermentation period and cell numbers due to greater utilization of the RS by the microbial cells.

No protease or amylase activity levels were observed on day 0 (Fig. 2C and 2D). The protease activity levels in all the fermented samples, especially at 20 g RS/kg sample without N addition, increased and reached their maxima of 49.41 U/g sample (d.b.) on day 5, as discussed above-this value was higher than those produced by the other fermented samples. The increase in N concentration could have generated regulatory responses that led to the expression of protease genes, affecting the reduction of enzymes (Dwitaviani et al., 2024), as illustrated by the decreases in the number of cells for the same fermentation period. Nevertheless, the protease activity levels of almost all the fermented samples decreased after day 5 because of nutrient reductions during fermentation, resulting in unfavorable conditions for microbial growth and producing a subsequent decrease in enzyme production. The amylase activity level of the fermented samples at 20 g RS/kg sample with added 5 g N/kg sample, 10 g N/kg sample, 15 g N/kg sample, 20 g N/kg sample and 25 g N/kg sample reached their maxima during days 3 and 5, with most of these activity levels remaining relatively constant thereafter. The amylase activity of the fermented sample at 20 g RS/kg sample without any added N increased continuously until the end of fermentation and was higher than those of the other conditions. High N concentrations might have affected the growth of microorganisms and suppressed amylolytic activity, causing enzyme inhibition (Sharma et al., 2012; Dash et al., 2015). There was no protease or amylase activity in the negative control sample at the end of fermentation.

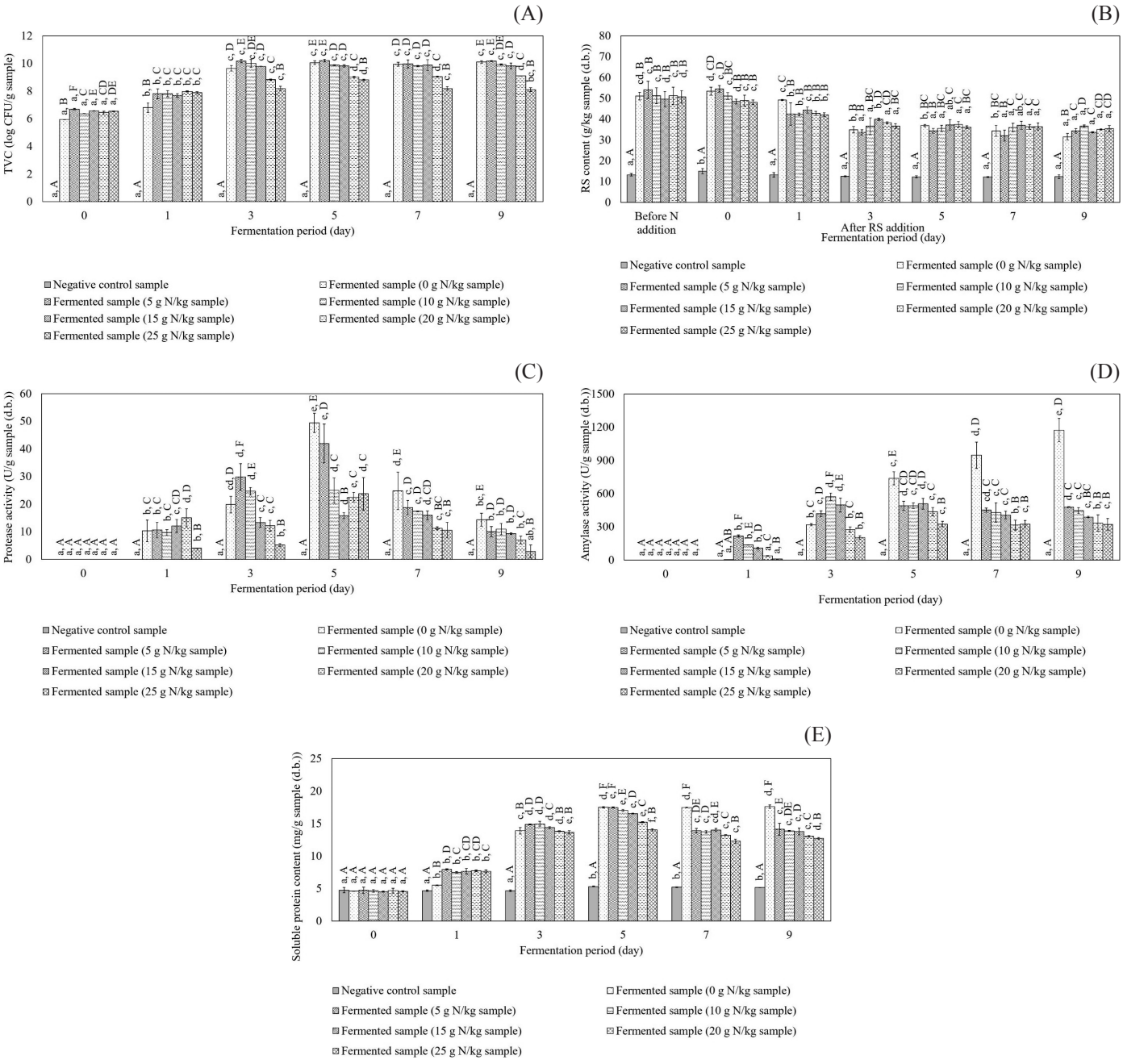

Fig. 2 Changes in total viable count (TVC), reducing sugar (RS) contents, enzyme activity levels and soluble protein contents of palm kernel cake with the addition of RS at 20 g RS/kg sample and different additions of N undergoing solid state fermentation at 37°C for 9 d: (A) TVC; (B) RS content; (C) protease activity; (D) amylase activity; (E) soluble protein content. Bars with the same lowercase letters within the same sample and the same uppercase letters within the same fermentation time are not significantly ($p \geq 0.05$) different.

Table 3 Changes in percentage of moisture contents of palm kernel cake with additions of RS at 20 g RS/kg sample and different levels of N undergoing solid state fermentation at 37°C for 9 d

Sample	Day 0	Day 1	Day 3	Day 5	Day7	Day 9
Negative control sample	59.53±2.59$^{a, A}$	58.29±3.60$^{a, A}$	58.41±0.49$^{a, A}$	57.15±1.07$^{a, A}$	58.48±0.56$^{a, A}$	57.78±1.01$^{a, A}$
Fermented sample (0 g N/kg sample)	59.39±1.05$^{a, A}$	60.58±0.76$^{a, ABC}$	63.79±0.32$^{b, D}$	65.86±1.23$^{c, D}$	66.95±0.54$^{c, E}$	66.78±1.62$^{c, E}$
Fermented sample (5 g N/kg sample)	60.77±0.35$^{a, A}$	63.11±0.05$^{b, C}$	62.70±0.04$^{b, CD}$	63.09±0.60$^{b, C}$	60.37±1.24$^{a, B}$	60.99±0.50$^{a, BC}$
Fermented sample (10 g N/kg sample)	59.13±0.91$^{a, A}$	61.65±0.23$^{b, BC}$	63.55±0.50$^{cd, D}$	63.53±1.08$^{cd, C}$	62.59±0.71$^{bc, CD}$	64.56±1.13$^{d, D}$
Fermented sample (15 g N/kg sample)	58.89±0.54$^{a, A}$	61.53±0.53$^{b, BC}$	62.84±0.39$^{c, CD}$	62.57±0.78$^{c, BC}$	62.81±0.32$^{c, D}$	59.28±0.73$^{a, AB}$
Fermented sample (20 g N/kg sample)	58.34±1.37$^{a, A}$	60.23±0.73$^{b, ABC}$	61.56±0.53$^{bc, BC}$	62.38±0.26$^{c, BC}$	61.37±0.83$^{bc, BC}$	61.58±0.27$^{bc, C}$
Fermented sample (25 g N/kg sample)	58.63±0.58$^{a, A}$	59.89±1.22$^{ab, AB}$	60.60±1.60$^{b, B}$	61.06±0.07$^{b, B}$	60.86±0.21$^{b, B}$	60.39±0.94$^{b, BC}$

Values (mean ± SD) with the same lowercase superscript letters within the same sample and the same uppercase superscript letters within the same fermentation period are not significantly ($p \geq 0.05$) different.

Table 4 Changes in pH of palm kernel cake with additions of reducing sugar (RS) at 20 g RS/kg sample and different levels of N undergoing solid state fermentation at 37°C for 9 d

Sample	Day 0	Day 1	Day 3	Day 5	Day7	Day 9
Negative control sample	6.55±0.04$^{ab, B}$	6.84±0.04$^{b, B}$	6.47±0.01$^{a, A}$	6.51±0.04$^{ab, A}$	6.48±0.01$^{a, A}$	6.42±0.06$^{a, A}$
Fermented sample (0 g N/kg sample)	6.86±0.04$^{a, A}$	6.76±0.59$^{a, A}$	7.50±0.06$^{c, B}$	6.88±0.15$^{b, B}$	7.23±0.28$^{c, B}$	7.41±0.42$^{c, B}$
Fermented sample (5 g N/kg sample)	7.10±0.03$^{a, C}$	7.53±0.08$^{b, C}$	8.34±0.01$^{d, D}$	8.37±0.04$^{d, C}$	8.23±0.04$^{c, C}$	8.17±0.08$^{c, C}$
Fermented sample (10 g N/kg sample)	7.56±0.07$^{a, D}$	7.83±0.18$^{b, CD}$	8.18±0.07$^{c, C}$	8.57±0.06$^{d, D}$	8.56±0.06$^{d, D}$	8.51±0.06$^{d, D}$
Fermented sample (15 g N/kg sample)	7.74±0.04$^{a, E}$	7.98±0.04$^{b, DE}$	8.54±0.04$^{cd, E}$	8.60±0.07$^{d, D}$	8.61±0.00$^{d, D}$	8.51±0.05$^{c, D}$
Fermented sample (20 g N/kg sample)	7.87±0.03$^{a, F}$	7.96±0.02$^{b, DE}$	8.52±0.01$^{c, E}$	8.51±0.10$^{c, CD}$	8.59±0.02$^{cd, D}$	8.61±0.02$^{d, D}$
Fermented sample (25 g N/kg sample)	8.15±0.01$^{a, G}$	8.30±0.01$^{b, E}$	8.52±0.02$^{d, E}$	8.40±0.08$^{c, C}$	8.49±0.03$^{cd, D}$	8.49±0.08$^{cd, D}$

Values (mean ± SD) with the same lowercase superscript letters within the same sample and the same uppercase superscript letters within the same fermentation period are not significantly ($p \geq 0.05$) different.

The soluble protein contents of all the fermented samples increased slightly and reached their maxima in the range 14.03–17.51 mg/g sample (d.b.) at day 5 (Fig. 2E). After that, the soluble protein contents of these samples decreased until day 9, except for the fermented samples containing 20 g RS/kg sample without N addition, which had unchanged content. On day 5, the maximum soluble protein contents of the fermented sample containing 20 g RS/kg sample without N addition (17.51 mg/g sample (d.b.)) and the fermented sample containing 20 g RS/kg sample with added 5 g N/kg sample (17.47 mg/g sample (d.b.)) were slightly higher than those of other fermented samples, though their contents were not significantly different. The soluble protein content of the negative control sample (4.65–5.33 mg/g sample (d.b.)) did not change significantly throughout the fermentation period.

The fermented sample at 20 g RS/kg sample without N addition (0 g N/kg sample) had similar maximum viable counts and soluble protein contents to those of the 20 g RS/kg samples with added 1 g N/kg sample, 5 g N/kg sample, 10 N/kg sample or 15 g N/kg sample. The maximum protease activity of this fermented sample on day 5 was higher than those for the other conditions and also 3.39-fold higher than that obtained from the fermented sample at 20 g RS/kg sample with added 1 g N/kg sample. Although the maximum amylase activity of this fermented sample was on day 9, the overall results indicated that the fermentation of PKC together with RS at 20 g RS/kg sample without N addition for 5 d was sufficient to produce cell counts sufficient to be referred to as probiotics. Notably, the amylase activity of the fermented sample at 20 g RS/kg sample without N addition on day 5 was 739.13 U/g sample (d.b.) and much higher than that of the fermented sample at 20 g RS/kg sample with added 1 g N/kg sample, representing an increase of 5.33-fold. Therefore, the fermentation of PKC with the addition of only RS at 20 g RS/kg sample on day 5 was selected as the most suitable fermentation process, which had a TVC of 10.05 log CFU/g sample (1.16×10^{10} CFU/g sample), a soluble protein content of 17.51 mg/g sample (d.b.) and protease and amylase activity levels of 49.41 U/g sample (d.b.) and 739.13 U/g sample (d.b.), respectively.

Proximate chemical compositions

The proximate chemical components of PKC (untreated sample) and fermented PKC at 20 g RS/kg sample without N addition were determined on a dry matter basis, as illustrated in Table 5. The crude protein content of fermented PKC at 20 g RS/kg sample without N addition (20.98%) was significantly higher than that of only PKC (13.79%), indicating that this fermented sample could increase the crude protein content 1.52-fold due to the breakdown of complex ingredients into amino acids by microbial enzymes, as well as the increase in the number of cells enhancing protein biomass (Wang et al., 2023). The crude fiber of the fermented PKC (25.40%) was slightly reduced by 0.94-fold compared to PKC (27.11%) and was allied with hemicellulose degradation in the fermented PKC from 29.48% to 22.14% (w/w). These results were similar to those reported by Wattanakul et al. (2012), which indicated that the fermentation of palm kernel meal (PKM) using SSF with mixed microorganisms (photosynthetic bacteria, lactic acid bacteria, nitrogen-fixing bacteria, yeast and *Bacillus* sp.) resulted in an increase in the crude protein content from 13.13% to 15.86% and a reduction of crude fiber content from 38.74% to 24.62% compared to only PKM. The ash content of the fermented sample (8.65%) was higher than that achievable from only PKC (4.90%) by 1.77-fold, due to the degradation of organic compounds during fermentation (Marini et al., 2008). However, the fat contents of the fermented PKC and PKC were not significantly different.

Table 5 Proximate chemical compositions of palm kernel cake (PKC) and fermented PKC by *B. velezensis* at 20 g reducing sugar (RS)/kg sample without N addition and fermentation for 5 d

Chemical component (% weight per weight)	PKC	Fermented PKC by *B. velezensis* at 20 g RS/kg sample without N addition and fermentation for 5 d
Crude protein	13.79±0.78[a]	20.98±0.71[b]
Crude fiber	27.11±0.31[b]	25.40±0.24[a]
Ash	4.90±0.09[a]	8.65±0.04[b]
Fat	5.94±0.38[a]	6.39±0.14[a]
N-free extract	48.26±1.36[b]	38.59±1.60[a]

Values (mean ± SD) in each row with the same lowercase superscript letters are not significantly ($p \geq 0.05$) different.

Conclusions

The SSF of PKC using *Bacillus velezensis* and adding RS from molasses and N from urea as supplements improved microbial growth and biological production based on the TVC, protein content and enzyme activity levels of the fermented samples. The addition of only 20 g RS/kg sample without N addition for 5 d fermentation provided suitable fermentation conditions based on the high levels of TVC 10.05 log CFU/g sample or 1.16×10^{10} CFU/g sample), soluble protein content (17.51 mg/g sample (d.b.)) and enzyme activity levels of protease and amylase (49.41 U/g sample (d.b.) and 739.13 U/g sample (d.b.), respectively). The crude protein content of PKC for these selected optimal conditions increased 1.52-fold (from 13.79% to 20.98% after 5 d of fermentation), while the crude fiber content of the fermented PKC (25.40%) slightly decreased 0.94-fold compared to PKC (27.11%). Overall, the conventional utilization of PKC as an alternative protein source in animal feed using solid state fermentation of PKC should be of interest to improve the nutritional value of fermented PKC and also for its application for probiotic production.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

The National Research Council of Thailand provided financial support for the study titled “Improving microbial growth and biological productions of fermented palm kernel cake through solid state fermentation using *Bacillus velezensis* with addition of reducing sugar from molasses and nitrogen from urea as supplements”.

References

Afrisham, S., Badoei-Dalfard, A., Namaki-Shoushtari, A., Karami, Z. 2016. Characterization of a thermostable, $CaCl_2$-activated and raw-starch hydrolyzing alpha-amylase from *Bacillus licheniformis* AT70: Production under solid state fermentation by utilizing agricultural wastes. J. Mol. Catal. B Enzym. 132: 98–106. doi.org/10.1016/j.molcatb.2016.07.002

Alrumman, S., Mostafa, Y.S.M., Al-Qahtani, S., Taha, T.H.T. 2018. Hydrolytic enzyme production by thermophilic bacteria isolated from Saudi hot springs. Open Life Sci. 13: 470–480. doi: 10.1515/biol-2018-0056

American Society for Testing and Materials D3174-12. 2012. Standard test method for ash in the analysis sample of coal and coke from coal. In: Annual Book of ASTM Standards. West Conshohocken, PA, USA.

American Public Health Association. 2017. Standard methods for the examination of water and wastewater. In: American Public Health Association, 23rd ed. Washington, DC, USA.

Association of Official Analytical Chemists. 2012. Official Methods of Analysis, 19th ed. Gaithersburg, MD, USA.

Azizi, M.N., Loh, T.C., Fo, H.L., Chung, E.L.T. 2021. Is palm kernel cake a suitable alternative feed ingredient for poultry? Anim. 11: 1–15. doi.org/10.3390/ani11020338

Banik, S., Biswas, S., Karmakar, S. 2018. Extraction, purification, and activity of protease from the leaves of *Moringa oleifera*. F1000Research. 7: 1–13. doi.org/10.12688/f1000research.15642.1

Bano, S., Qader, S.A.U., Aman, A., Syed, M.N., Azhar, A. 2011. Purification and characterization of novel α-amylase from *Bacillus subtilis* KIBGE HAS. AAPS Pharm. Sci. Tech. 12: 255–261. doi.org/10.1208/s12249-011-9586-1

Bello, B., Mustafa, S., Tan, J.S., Ibrahim, T.A.T., Tam, Y.J., Arif, A.B., Manap, M.Y., Abbasiliasi, S. 2018. Evaluation of the effect of soluble polysaccharides of palm kernel cake as a potential prebiotic on the growth of probiotics. 3 Biotech. 8: 1–14. doi.org/10.1007/s13205-018-1362-4

Berovic, M., Podgornik, B.B. 2016. Cultivation of medicinal fungi in bioreactors. In: Petre, M. (Eds.). Mushroom Biotechnology: Developments and Applications. Academic Press. London, England, pp. 155–171.

Boratyński, F., Szczepańska, E., Grudniewska, A., GniÍka, R., Olejniczak, T. 2018. Improving of hydrolases biosythesis by solid state fermentation of *Penicillium camemberti* on rapeseed cake. Sci. Rep. 8: 1–9. doi.org/10.1038/s41598-018-28412-y

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254. doi.org/10.1016/0003-2697(76)90527-3

Chai, J.M., Adnan, A. 2018. Effect of different nitrogen source combinations on microbial cellulose production by *Pseudomonas aeruginosa* in batch fermentation. In: Proceedings of The International Fundamentum Sciences Symposium Conference. Terengganu, Malaysia, pp. 1–9.

Cui, Y., Zhu, J., Li, P., et al. 2024. Assessment of probiotic *Bacillus velezensis* supplementation to reduce *Campylobacter jejuni* colonization in chickens. Poult. Sci. 103: 103897. doi.org/10.1016/j.psj.2024.103897

Dash, B.K., Rahman, M.M., Sarker, P.K. 2015. Molecular identification of a newly isolated *Bacillus subtilis* BI19 and optimization of production conditions for enhanced production of extracellular amylase. Biomed. Res. Int. 2015: 1–9. doi.org/10.1155/2015/859805

Dwitaviani, R., Agustien, A., Djamaan, A. 2024. Optimizing carbon and nitrogen for protease production in endophytic bacteria from Mandeh, Pesisir Selatan. Int. J. Progress. Sci. Technol. 44: 103–110. doi.org/10.52155/Ijpsat.v44.2.6179

Egenuka, F.C., Ndubuisi, N.A., Iwujii, P.C., Okere, P.C., Okeudo, N.J. 2018. Effects of high dietary levels of palm kernel cake on performance and cost of production of starter broilers. In: Proceeding of 43rd Annual Conference of the Nigerian Society for Animal Production. Federal University of Technology, Owerri, Nigeria, pp. 403–405.

Epstein, E. 2017. The Science of Composting, 1st ed. CRC Press. Boca Raton, FL, USA, pp. 647.

Gracia, M.I., Aranibar, M., Lazaro, R., Medel, P., Mateos, G.G. 2003. Alpha-amylase supplementation of broiler diets based on corn. Poult. Sci. 82: 436–442. doi.org/10.1093/ps/82.3.436

Heidari, P., Kordestany, A., Ghazanfari, M.H. 2011. An experimental investigation of parameters affecting oil recovery efficiency alteration during a microbially aided water flooding process. Petrol. Sci. Technol. 29: 2507–2519. doi.org/10.1080/10916461003699200

Jenkins, C., Ling, C.L., Ciesielczuk, H.L., Lockwood, J., Hopkins, S., McHugh, T.D., Gillespie, S.H., Kibbler, C.C. 2012. Detection and identification of bacteria in clinical samples by 16S rRNA gene sequencing: comparison of two different approaches in clinical practice. J. Med. Microbiol. 61: 483–488. doi.org/10.1099/jmm.0.030387-0

Jung, S.-J. 2016. Quality and functional characteristics of kimchi made with organically cultivated young Chinese cabbage, J. Ethn. Foods. 3: 150–158. doi.org/10.1016/j.jef.2016.05.003

Kaczmarek, S.A., Rogiewicz, A., Mogielnicka, M., Rutkowski, A., Jones, R.O., Slominski, B.A. 2014. The effect of protease, amylase, and nonstarch polysaccharide-degrading enzyme supplementation on nutrient utilization and growth performance of broiler chickens fed corn-soybean meal-based diets. Poult. Sci. 93: 1745–1753. doi.org/10.3382/ps.2013-03739

Khalid, F., Khalid, A., Fu, Y., Hu, Q., Zheng, Y., Khan, S., Wang, Z. 2021. Potential of *Bacillus velezensis* as a probiotic in animal feed: A review. J. Microbiol. 59: 627–633. doi.org/10.1007/s12275-021-1161-1

Li, J., Tao, W., Yue, S., Yuan, Z., Li, S. 2023. Adaptive laboratory evolution of *Bacillus subtilis* 168 for efficient production of surfactin using NH_4Cl as a nitrogen source. Fermentation, 9: 1–14. doi.org/10.3390/fermentation9060525

Lomthong, T., Areesirisuk, A., Suphan, S., Panyachanakul, T., Krajangsang, S., Kitpreechavanich, V. 2021. Solid state fermentation for poly (L-lactide)-degrading enzyme production by *Laceyella sacchari* LP175 in aerated tray reactor and its hydrolysis of poly (lactide) polymer. Agr. Nat. Resour., 55: 147–152. doi.org/10.34044/j.anres.2021.55.1.19

Lunsin, R., Duanyai, S., Pilajun, R., Duanyai, S., Sombatsri, P. 2018. Effect of urea- and molasses-treated sugarcane bagasse on nutrient composition and *in vitro* rumen fermentation in dairy cows. Agr. Nat. Resour. 52: 622–627. doi.org/10.1016/j.anres.2018.11.010

Marini, A.M., Ayub, M.Y., Salam, B.A., Hadijah, H., Azahan, E.A.E., Tarmizi, S.A. 2008. Protein quality of *Aspergillus niger*-fermented palm kernel cake. J. Trop. Agric. Food Sci. 36: 227–237.

Marinova, V.Y., Rasheva, I.K., Kizheva, Y.K., Dermenzhieva, Y.D., Hristova, P.K. 2019. Microbiological quality of probiotic dietary supplements. Biotechnol. Biotechnol. Equip. 33: 834–841. doi.org/10.1080/13102818.2019.1621208

Morohashi, Y., Tomita, T. 2013. Protein trafficking and maturation regulate intramembrane proteolysis. Biochim. Biophys. Acta - Biomembr. 1828: 2855-2861. doi.org/10.1016/j.bbamem.2013.06.001

Niazifar, M., Besharati, M., Jabbar, M., Ghazanfar, S., Asad, M., Palangi, V., Eseceli, H., Lackner, M. 2024. Slow-release non-protein nitrogen sources in animal nutrition: A review. Heliyon. 10: 1–11. doi.org/10.1016/j.heliyon.2024.e33752

Nusrat, A., Rahman, S.R. 2007. Comparative studies on the production of extracellular α-amylase by three mesophilic *Bacillus* isolates. Bangladesh J. Microbiol. 24: 129–132. doi.org/10.3329/bjm.v24i2.1257

Ojo-Omoniyi, O.A., Moro, D.D., Afolab, O.B. 2024. Microbial Proteases: Sources, Significance and Industrial Applications. Int. J. Curr. Microbiol. App. Sci., 13: 1–23. doi.org/10.20546/ijcmas.2024.1306.001

Oxenboll, K.X., Pontoppidan, K. Fru-Nji, F. 2011. Use of a protease in poultry feed offers promising environmental benefits. Int. J. Poult. Sci. 10: 842–848. doi.org/10.3923/ijps.2011.842.848

Pranay, K., Padmadeo, S.R., Prasad, B. 2019. Production of amylase from *Bacillus subtilis* sp. strain KR1 under solid state fermentation on different agrowastes. Biocatal. Agric. Biotechnol. 21: 1–8. doi.org/10.1016/j.bcab.2019.101300

Qiu, K., Chen, J., Zhang, G., Chang, W., Zheng, A., Cai, H., Liu, G., Chen, Z. 2023. Effects of dietary crude protein and protease levels on performance, immunity capacity, and AA digestibility of broilers. Agr. 13: 1–12. doi.org/10.3390/agriculture13030703

Saqib, A.A.N., Whitney, P.J. 2011. Differential behaviour of the dinitrosalicylic acid (DNS) reagent towards mono- and di-saccharide sugars. Biomass Bioenerg. 35: 4748–4750. doi.org/10.1016/j.biombioe.2011.09.013

Sathitkowitchai, W., Nitisinprasert, S., Keawsompong, S. 2018. Improving palm kernel cake nutrition using enzymatic hydrolysis optimized by Taguchi method. 3 Biotech. 8: 1–7. doi.org/10.1007/s13205-018-1433-6

Šelo, G., Planinić, M., Tišma, M., Tomas, S., Koceva Komlenić, D., Bucić-Kojić, A. 2021. A comprehensive review on valorization of agro-food industrial residues by solid-state fermentation. Foods. 10: 927. doi.org/10.3390/foods10050927

Sharma, N., Vamil, R., Ahmad, S., Agarwal, R. 2012. Effect of different carbon and nitrogen sources on α-amylase production from *Bacillius amyloliquefaciens*. Int. J. Pharm. Sci. Res. 3: 1161–1163.

Sjölin, M., Sayed, M., Thuvander, J., Lipnizki, F., Hatti-Kaul, R., Wallberg, O. 2022. Effect of membrane purification and concentration of sucrose in sugar beet molasses for the production of 5-hydroxymethylfurfural. Chem. Eng. Res. Des. 179: 365–373. doi.org/10.1016/j.cherd.2022.01.007

Stefanello, C., Vieira, S.L., Rios, H.V., Simões, C.T., Ferzola, P.H., Sorbara, J.O.B., Cowieson, A.J. 2017. Effects of energy, α-amylase, and β-xylanase on growth performance of broiler chickens. Anim. Feed Sci. Technol. 225: 205–212. doi.org/10.1016/j.psj.2020.09.007

Urban, J., Jaworski, S., Lange, A., Bień, D., Matuszewski, A., Michalczuk, M. 2023. Effects of the addition of crude fibre concentrate on performance, welfare and selected caecal bacteria of broilers. Anim. 13: 3883. doi.org/10.3390/ani13243883

Vinale, F. and Balestrieri, M.L. 2020. Recent Advances in Natural Products Chemistry Related to Metabolites and Microbiomes, (Eds.). MDPI. Basel, Switzerland, pp. 198.

Wang, J., Jiang, Q., Huang, Z., Wang, Y., Roubik, H., Yang, K., Cai, M., Sun, P. 2023. Solid-state fermentation of soybean meal with edible mushroom mycelium to improve its nutritional, antioxidant capacities and physicochemical properties. Ferment. 9: 1–14. doi.org/10.3390/fermentation9040322

Wang, T. 2012. Evaluation of enzyme activity and fiber content of soybean cotyledon fiber and distiller's dried grains with solubles by solid state fermentation. Appl. Biochem. Biotechnol. 167: 109–121. doi.org/10.1007/s12010-012-9665-0

Wang, Y., Yu, J., Li, Q., Zhang, J., Naseem, S., Sun, B., Tang, L., Choi, S., Li, X. 2023. Screening the carbon source type in solid-state fermentation with *Phanerochaete chrysosporium* to improve the forage value of corn straw and rice straw. Anim. 13: 1–13. doi.org/10.3390/ani13050888

Wattanakul, W., Thongprajukaew, K., Hahor, W., Suanyuk, N. 2021. Optimal replacement of soybean meal with fermented palm kernel meal as protein source in a fish meal-soybean meal-based diet of sex reversed red tilapia (*Oreochromis niloticus* × *O. mossambicus*). Anim. 11, 2287. doi: 10.3390/ani11082287

Williams, P.T. 2013. Waste Treatment and Disposal, 2nd ed. John Wiley & Sons, Chichester, England, pp. 424.

Yadav, J.K., Prakash, V. 2011. Stabilization of α-amylase, the key enzyme in carbohydrates properties alterations, at low pH, Int. J. Food Prop. 14: 1182–1196. doi.org/10.1080/10942911003592795

Yin, D., Yin, X., Wang, X., Lei, Z., Wang, M., Guo, Y., Aggrey, S.E., Bnei, W. Yuan, J. 2018. Supplementation of amylase combined with glucoamylase or protease changes intestinal microbiota diversity and benefits for broilers fed a diet of newly harvested corn. J. Anim. Sci. Biotechnol. 9: 1–13. doi.org/10.1186/s40104-018-0238-0

Zeng, J., Huang, W., Tian, X., Hu, X., Wu, Z. 2021. Brewer's spent grain fermentation improves its soluble sugar and protein as well as enzymatic activities using *Bacillus velezensis*. Process Biochem. 111: 12–20. doi.org/10.1016/j.procbio.2021.10.016

Zhu La, A.L.T., Wen, Q., Xiao, Y., Hu, D., Liu, D., Guo, Y., Hu, Y. 2024. A New *Bacillus velezensis* strain CML532 improves chicken growth performance and reduces intestinal *Clostridium perfringens* colonization. Microorganisms. 12: 771. doi.org/10.3390/microorganisms12040771