ASIA-PACIFIC JOURNAL OF MOLECULAR BIOLOGY AND BIOTECHNOLOGY

AsPac J. Mol. Biol. Biotechnol. 2025
ISSN: 0128-7451, eISSN: 2672-7277
Vol. 33 (1): 108-118
https://doi.org/10.35118/apjmbb.2025.033.1.12

RESEARCH ARTICLE

Purification and characterisation of Bile-salt activated lipase (BSAL) enzyme from bovine (*Bos taurus*) pancreas

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Abstract
Bile salt-activated lipase (BSAL) is one of the pancreatic lipases that plays a critical role in the digestion and absorption of dietary fats. Objective: This study aimed to purify bile salt-activated lipase (BSAL) from bovine (*Bos taurus*) pancreatic tissue. Methods: Bovine pancreas was freshly collected from Abattoir Complex, Department of Veterinary Services, Shah Alam, Malaysia. The fats were removed by trimming grossly visible fat, and extraction was performed using organic solvents. The BSAL was purified by anion exchange chromatography and sent for protein identification by liquid chromatography-mass spectrometry (LC-MS/MS). Successful purification of bovine BSAL was visualised as a single protein band on sodium dodecyl sulphate-polyacrylamide (SDS) gel, which LC-MS/MS confirmed as a bovine BSAL (Accession number – P30122) with a molecular mass of 65.12 kDa and calc pI of 5.57. Peptide identification based on the MS spectrum found 200 predictive peptides, ten sequences with bovine BSAL peptide characteristics. The selected predictive peptide sequences have a molecular mass of 1104.60 - 3378.94 Da with Qvality q-value greater than 0.01 and XCorr Sequest HT value ranging from 2.6 to 6.8. The specific lipolytic enzyme activity of bovine BSAL was comparable with the positive control, as measured using lipase assay. In conclusion, the results of this study indicate the effectiveness of bovine BSAL purification by anion exchange chromatography from fresh pancreatic tissue and may have the potential for further Halal pharmaceuticals and medical applications.

Keywords: bile salt-activated lipase (BSAL), enzyme purification, halal enzyme, lipase, pancreas, pancreatic enzyme

INTRODUCTION

There are three major classes of digestive enzymes, namely, protease, amylase, and lipase (Hou & Shimada, 2009), are produced in the pancreas to facilitate food digestion (Oliveira et al., 2024; Peter et al., 2001). These enzymes can also be found in other sources, such as bacteria, fungi, and eukaryotes (Park & Park, 2022; Souza & Magalhães, 2010). Lipases are the third most utilised enzyme and biocatalysts in medicine, pharmaceutical, food, as well as detergent industries, after proteases and amylases (Vardar-Yel et al., 2024; Yu et al., 2016). This class of enzymes is responsible for catalysing the hydrolysis of triacylglycerols to glycerol and fatty acids essential (Hou & Shimada, 2009) to provide the highest dietary source of calories (Oliveira et al., 2024). Lipase has become of remarkable interest to many researchers as most nutritional problems are secondary to the improper digestion of dietary fats (Szkopek et al., 2024). Two major lipase clusters that work synergistically are gastric and pancreatic lipases. The exocrine pancreas synthesises and secures several lipolytic enzymes, which work with gastric lipase to achieve complete dietary fat digestion and absorption (Li et al., 2007). The gastric lipase contributes

Received: 24 October 2024
Accepted: 31 December 2024
Published: 31 March 2025
Published by: Malaysian Society for Molecular Biology and Biotechnology (MSMBB)

approximately 10-30% of lipolytic activities, which participates in the early stage of lipolysis to release fatty acids in the stomach alongside bile salt-dependent lipase and pancreatic colipase-dependent lipases (Oliveira et al., 2024).

Bile salt-activated lipase (BSAL, EC 3.1.1.13) is one of the pancreatic lipases that plays a critical role in the digestion and absorption of dietary fats. It is also known as bile salt-stimulated lipase, carboxyl ester lipase, cholesterol esterase, pancreatic lysophospholipase, and bile salt-dependent lipase (Hui & Howles, 2002; Lindquist et al., 2023; Lombardo, 2001; Wang & Hartsuck, 1993; Wang et al., 1997). It is a lipase secreted from the vertebrate exocrine pancreas into the intestine and is essential for lipid digestion (Wang et al., 1997). The presence of bile salt is crucial in activating BSAL mechanisms for digestion and absorption as a bio-surfactant in the gastrointestinal tract, particularly to address low solubility and drug delivery transportation (Maldonado-Valderrama et al., 2011; Mukhopadhyay & Maitra, 2004).

BSAL has a comprehensive specificity and hydrolyses a variety of different substrates (Hernell & Olivecrona, 1974; Lindquist et al., 2012, 2023), classified as significant lipids (dietary fat and triacylglycerol) and minor lipids (fat-soluble vitamin esters and cholesterol esters digestion) (Wang & Kloer, 1983; Wang et al., 1999). A sound lipolytic system is needed for BSAL, along with other pancreatic lipolytic enzymes and gastric lipase, to act in concert for complete dietary lipid digestion (Bruneau et al., 2000). Drastic reduction of BSAL leads to the development of many diseases, such as pancreatic cancer, necrotising pancreatitis, and chronic pancreatitis, affecting the gastrointestinal tract in the digestive system (Lombardo, 2001).

BSAL is mainly isolated from pancreatic tissues and has also been reported to be extracted from the liver and mammary gland (Gjellesvik et al., 1992). BSAL was also reported to have considerable lipase activity in human milk, significantly contributing to milk lipid utilisation in newborns (Christie et al., 1991). High BSAL content in breast milk significantly increases the fat digestion capacity of newborns during the first few months after birth due to immature pancreatic development and limited pancreatic lipase secretion (Koh et al., 2020; Manson & Weaver, 1997). The purification of pancreatic BSAL has been attempted from several sources, including humans (Abouakil et al., 1988; Manson & Weaver, 1997; Wang & Kloer, 1983), porcine (Labow et al., 1983; Momsen & Brockman, 1977; Rudd et al., 1987), bovine (Chen et al., 1998; Tanaka et al., 1999), rats (Abouakil et al., 1993; Mendes et al., 2012), and fish (Gjellesvik et al., 1992). The isolation of BSAL from the pancreatic tissue is more complicated due to proteolytic digestion during the purification process (Wang & Hartsuck, 1993). However, pancreatic lipase from animal sources has been reported to have more excellent stability with high activity (Mendes et al., 2012). In addition, pancreatic enzymes originating directly from the pancreas of mammals are naturally suitable for digestion and have been studied for their application in the medical and pharmaceutical fields as the animal-origin enzyme is known to possess high therapeutic potential (Headon & Walsh, 1994). Although BSAL can be isolated from milk, pancreatic BSAL is also crucial as they have distinct physical and biochemical properties. Both milk BSAL and pancreatic BSAL are vital digestive enzymes and carry different responsibilities for fat breakdown and digestion of dietary fats within the lumen of the intestine, respectively. As for the clinical implications, milk BSAL and pancreatic BSAL objectively depend on the specific requirement and application. Obtaining the pancreatic BSAL can be significant for developing pancreatic disorder treatment. On the other hand, milk BSAL may be used in dietary formulas and nutritional supplements to enhance fat digestion (Koh et al., 2020). With biotechnological advancement, the extraction and purification of pancreatic enzymes from Halal sources can be achieved with lower production costs, thus fulfilling the demand for Halal products that are increasing globally.

Although the study on BSAL purification has previously received considerable attention, there has been no recent update on the methods related to the extraction and purification of BSAL, as well as the peptides sequence analysis by LC-MS/MS from the bovine pancreas. The research interest in BSAL has switched from purification to its application to induce smooth muscle cell proliferation (Augé et al., 2003), as well as *in vitro* angiogenic effects suggesting potential implications in vascular pathophysiology (Rebaï et al., 2005).

The Bovidae family, including domestic cattle, is widely distributed in Malaysia for the livestock agriculture sector, as beef is Malaysia's most popular source of red meat (Ariff et al., 2015). Nevertheless, higher meat production contributes to large amounts of waste as some animal organs are not fully utilised and are finally disposed of. This includes the pancreas, a non-edible organ produced in significant quantities by the meat industry and contributes towards waste disposal and potential pollution (Li et al., 2012). Thus, the bovine pancreas, a by-product of the meat industry, can be utilised as an alternative low-cost source of Halal digestive enzymes, particularly for lipase applications. In addition, the premier quality and Halal-certified products, including medicine and

health supplements, are gaining much attention from Muslim and non-Muslim consumers (Aziz et al., 2014). Furthermore, Halal pharmaceutical products are considered healthier, more hygienic (Sudarsono & Nugrowati, 2020), and generally better in quality, with a projected market value of $3.2 trillion by 2024 (American Halal Foundation, 2024). Combining Islamic principles with rigorous manufacturing standards and ethical considerations contributes to healthier and hygienic Halal pharmaceutical productions. The ethical Halal guidelines not only prohibit harmful substances that can lead to adverse health effects but also emphasise quality sources of ingredients and stringent hygiene production protocol to adhere to the Halal manufacturing standards (Herdiana et al., 2024).

In the pharmaceutical industry, lipases have been utilised as modulators (activators and inhibitors) to treat lifestyle diseases such as obesity (Szkopek et al., 2024) and show great promise in therapies (Chandra et al., 2020). Microbial lipase from bacteria, fungi, and yeast is the current primary source and is pivotal in various industries, including pharmaceuticals (Vardar-Yel et al., 2024; Vivek et al., 2022). Although many microbial lipases are currently available, they are still inadequate for fulfilling the requirements of most industries, specifically in medicine and pharmaceuticals, due to the possible variation in behaviour and functionality compared to animal lipase. Therefore, successful purification of BSAL from bovine pancreas can be suggested as an alternative substitute for porcine lipase to cater for Halal medical and pharmaceutical demands.

MATERIALS AND METHODS

Extraction and purification of bile salt activated lipase

The bovine pancreas collection and pancreatic crude extraction were done on the same day. The bovine pancreas was collected from freshly slaughtered animals from the Abattoir Complex, Department of Veterinary Services, Shah Alam, Malaysia, and stored on ice. The pancreatic crude extraction process was initiated immediately within 1 hour after the bovine pancreas collection. The bovine pancreas was firstly washed with tap water and then dissected free from adherent fat and connective tissues with a scalpel blade (Elamin et al., 2014). The pancreas was cut into smaller pieces on ice, and the tissue slices were washed with a 50 mM Tris pH 7.4 buffer containing 0.1 M sodium chloride (NaCl) to remove blood (Al-Ajlan & Bailey, 1999). Approximately 160 g of pancreas tissues were pooled and homogenised before being suspended in 20 ml of extraction buffer (50 mM Tris pH 7.4, 2 mM Ethylenediaminetetraacetic acid (EDTA) pH 8, 10 mM HEPES pH 7.4, 1% Triton X-100, and 150 mM NaCl) and vortexed for 10 mins before centrifugation at 4000 rpm for 2 mins. The supernatants were collected and added with 70% (v/v) ethanol, bringing a total of 25 ml. The mixture was vortexed for 10 secs, and 2 ml of 5 µg/ml of aprotinin (a protease inhibitor) was added to the samples. The samples were centrifuged at 14,000 rpm for 20 mins before collecting the supernatant. The supernatant was evaluated for absorbance at 280 nm and stored at -20°C until further use.

The purification step was performed at room temperature on the second day (within 24 hours) following the extraction. Bovine pancreatic BSAL were further purified by anion exchange chromatography on a pre-packed HiTrap Q Fast Flow column (bed height 1.6 cm, column diameter 2.5 cm, column volume 5 ml) using AKTA AvantTM chromatography system (GMI – USA). A 20 mM Tris pH 8 was used as the start buffer, and 20 mM Tris 1 M NaCl pH 8 was used as the elution buffer. The sample was filtered using 0.45 µm and 0.25 µm cellulose acetate pore membrane filters (Merck – Germany). A total of 200 ml filtered sample was applied to the column in the start buffer, and the column was washed until the absorbance monitored at 280 nm returned to baseline. The BSAL pancreatic enzymes were eluted using an elution buffer at a 2.4 ml/min flow rate, and 1.5 ml fractions were collected. The elution buffer composition was set up as a step gradient at 5%, 10%, 20%, 30%, 40%, 50%, and 100% NaCl to elute the targeted BSAL. During the elution steps, the absorbance readings were observed. The collection started at a 5% step gradient. The gradient step was based on the absorbance value, where the following step gradient was applied after the absorbance returned to the baseline approximately at 500 mAU. This is to ensure a better peak separation. Each fraction representing different peaks was run on 1-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis (1D SDS-PAGE) for molecular weight determination. Fractions that potentially have lipolytic activity based on the molecular weight were pooled and concentrated using a 30,000 MW and 50,000 MW pore-size concentrating device (MERCK).

Bile salt activated lipase protein and peptide identification

Protein concentration was measured at 280 nm using the NanoDrop® ND-1000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, US). The molecular mass of purified bovine pancreatic BSAL was estimated by 1D SDS-PAGE stained with Coomassie

Brilliant Blue stain (Invitrogen, US). Protein bands were further identified using liquid chromatography-mass spectrometry (LC-MS/MS). Before the LC-MS/MS procedure, trypsin gel digestion was carried out. Then, a total of 5 µl sample was injected into Dionex Ultimate 3000 RSLCnano Orbitrap Fusion (Thermo Fisher Scientific) with EASY-Spray Acclaim PepMapTM C18 100 A^0 (2 µm particle size, 50 µm id x 15 cm) separation column. The sample was eluted with LC-MS grade water, 0.1% formic acid (A), and ACN, 0.1% formic acid (B), at a 250 nL/min flow rate. The elution gradient was 5%-40% B for 91 min, 2 min to 85% B, 3 min at 85% B, 1 min at 5% B, and 4 min at 5% B. The full scan spectra were collected by orbitrap MS (OTMS) scan mass ranging from 310 – 1800 m/z with 50 ms maximum injection. The spectra were analysed by ion trap MS (ITMS) with 250 ms maximum injection. The LC-MS/MS data was analysed using Thermo ScientificTM Proteome DiscovererTM Software Version 2.1. The results were matched with the database downloaded from UniProt. The variable modifications were oxidation and deamination with fixed modification of carbamidomethyl. All peptides were validated using the percolator® algorithm, based on a q-value of less than 1% false discovery rate (FDR). The LC-MS/MS analysis was performed at the Proteomic Laboratory, Malaysian Genome and Vaccine Institute, Bangi, Malaysia.

Lipolytic enzyme assay

Lipase activity was evaluated through the conversion of 4-nitrophenyl Palmitate (pNPP) into p-nitrophenol (pNP) formation (Vo et al., 2022). The lipolytic activity of crude extracts and purified fractions from anion exchange chromatography were determined using the modified method from previous studies (Vardar Yel, 2021; Vo et al., 2022) alongside the commercial porcine pancreatic lipase (Sigma Aldrich, Germany) as a positive control. The pNPP calibration standard curve was prepared using serial dilution. The substrates were prepared by mixing pNPP with acetonitrile (ACN). The standard assay contained 10 µl of lipase sample, 150 µl of substrates, and 40 µl of 25mM sodium phosphate buffer pH 7. The 25mM sodium phosphate buffer pH 7 and ACN mixture was used as blank. The lipolytic assay was performed in a 96-well microplate with 200 µl reaction volume incubated at 25 °C. The experiments were repeated three times. One unit of lipase is defined as the amount of enzyme that will generate 1.0 µmole of pNP from the pNPP reaction per minute at 25 °C as measured at 405 nm using a SpectraMax iD5 Multi-mode Microplate reader (Molecular Devices, USA). The readings were taken periodically at 1, 5, 10, 15, 30, 45, 60, 90, 120 and 150 minutes.

RESULTS

Bovine pancreatic BSAL was successfully purified from the fresh pancreatic tissue by pancreatic tissue delipidation, protein extraction, and anion exchange chromatography. Figure 1 illustrates the elution pattern of anion exchange chromatography on the prepacked HiTrap Q Fast Flow column, developed by NaCl step gradient. A total of 17 fractions (25.5 ml) containing bovine BSAL were eluted at 10% NaCl concentration and labelled as peak B. Next, the molecular weight of the targeted enzyme was determined in fraction B, whereby a single protein band estimated at 60kDa (Figure 2, Lane 3) was subsequently observed with confirmed lipase activity. The band was excised and destained for LC-MS/MS protein identification, which confirmed it as a bovine pancreatic BSAL with a molecular mass of 65.12kDa and calc pI 5.57.

The bovine pancreatic BSAL (Accession number: P30122) with 30.9% protein coverage was identified as a hit by LC-MS/MS. The predictive peptide sequences of B determined by the LC-MS/MS were selected as potential bovine pancreatic BSAL based on the percolator® algorithm with a q-value of less than 1% false discovery rate (FDR). The peptide identification results showed that 199 predictive peptides were obtained (data not shown). In the current study, ten predictive peptides show similar characteristics to the same master protein of bovine pancreatic BSAL. The result obtained was confirmed with low FDR with Qvality q-value (>0.01), XCorr Sequest HT (>2.0), and percolator q-value Sequest HT value (>0.01). In general, the reported peptides contained one or no missed cleavage events. The peptide sequence, position in the master protein, charge, and molecular weight are listed in Table 1. Most of the predictive peptides have low molecular weight ranging from 1104.60 - 3378.94 Da and net charge of 2 to 3. Five of ten predictive peptide sequences undergo modifications during the sample handling process, as shown in Table 1, with frequent variable deamination and oxidation modifications, as well as the less frequent fixed carbamidomethyl modifications. Only two cysteine carbamido-methylation (CAM) modified sites were identified (Peptide amino acid 82-109 CLQATLTQDSTYGN EDCLYLNIWVPQGR, proposed CAM on C82 and C97; Peptide amino acid 234-256 AISQSGVG LCPWAIQQDPLFWAK, proposed CAM on C243).

The lipolytic activity was measured using the described lipase assay for all the crude and anion exchange chromatography samples. The pNPP standard curve was constructed to determine the lipase activity of bovine BSAL at different sequential purification stages, with the R2 value of 0.99 (Figure 3). Table 2 summarises the purification of bovine BSAL. The protein concentration of purified bovine pancreatic BSAL, as calculated from A280, was 6.83 mg/ml. The specific lipolytic activity was increased after anion exchange chromatography.

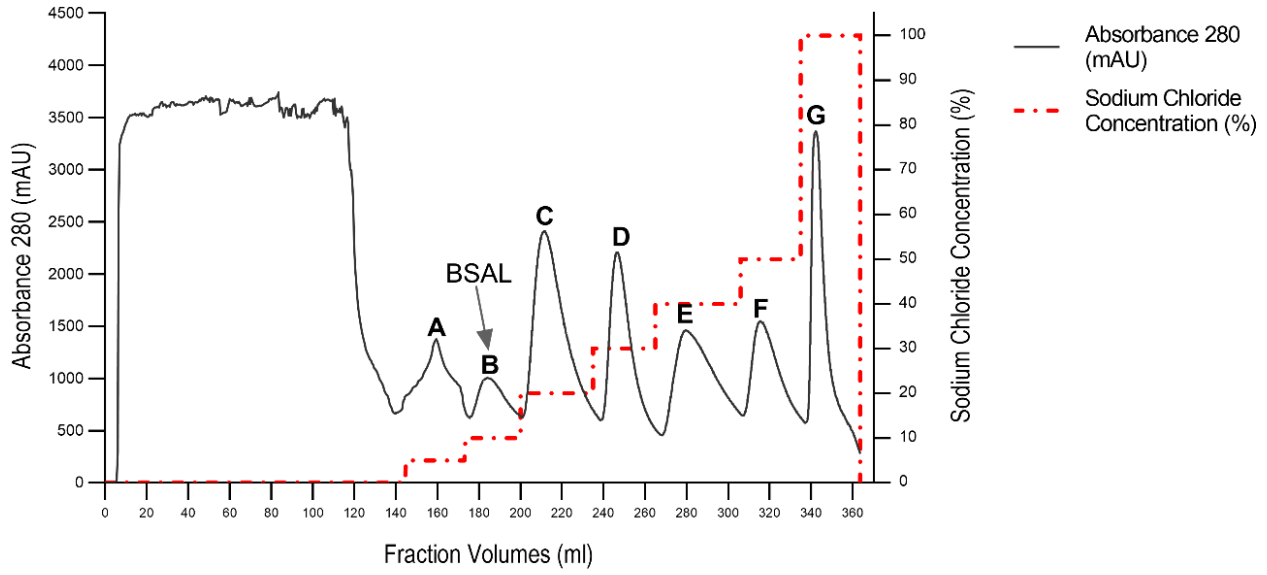

Figure 1. Anion-exchange chromatography of bovine pancreatic aqueous crude extract. The chromatogram represents the absorbance of crude extract at 280nm. The arrows marked peaks that showed the presence of bovine BSAL on the 1D SDS Page with lipolytic activity. Tubes were pooled at 10% NaCl concentration, indicating the presence of active BSAL. Column: Hi Trap Q Fast Flow column (pre-packed); flow rate: 2.4ml/min; mobile phase: 20mM Tris pH8, 20mM Tris NaCl pH8.

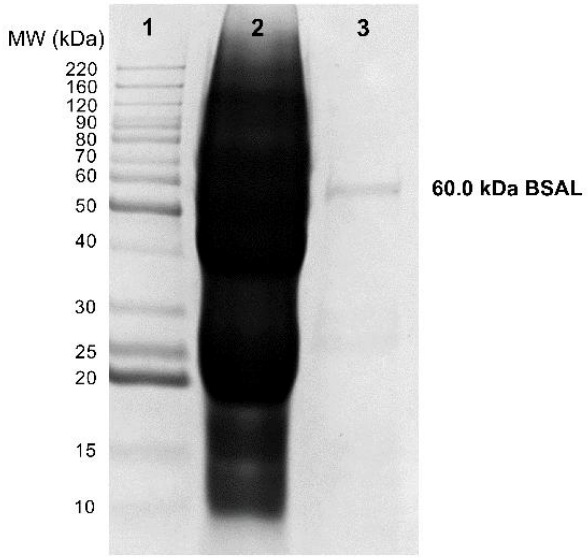

Figure 2. 1D SDS-PAGE (Bis-Tris 12%) of pure BSAL stained with Coomassie blue. Well 1: BenchmarkTM Unstained Protein Ladder; Well 2: Crude extract; Well 3: Purified BSAL obtained after Hi Trap Q Fast Flow chromatography.

Table 1. Protein identification and peptide sequence prediction of Bovine Pancreatic BSAL *via* LC-MS/MS.

No	Annotated Sequence	Confidence	Modifications	Charge	Position in Master Protein	Theo. MH+ [Da]	Qvality q-value	XCorr Sequest HT	Percolator q-Value Sequest HT
1	[R].TGDP**N**TGHSTVPA**N**WD PYTLEDD**N**YLEI**N**K.[Q]	High	1x Deaminated [N] 2x Deaminated [N14; N24]	2	492-521	3378.49	0	6.54	0
2	[R].VGPLGFLSTGDS**N**LPG**N** YGLWD**Q**H**M**AIAWVK.[R]	High	1xOxidation [M25] 1xDeamidated [N/Q] 2xDeamidated [N17; Q/N]	3	163-193	3361.62	0	6.64	0
3	[R].AIS**Q**SGVGL**C**PWAI**QQ**DP LFWAK.[R]	High	1xDeamidated [Q] 1xCarbamidomethyl [C10]	3	234-256	2572.30	0	5.76	0
4	[R].**C**L**Q**ATLT**Q**DSTYG**N**ED**C** LYL**N**IWVPQGR.[K]	High	1xDeamidated [Q/N] 2xCarbamidomethyl [C1; C17]	3	82-109	3315.54	0	3.56	0
5	[K].T**M**VDLETDILFLIPTK.[I]	High	1xOxidation [M2]	2	401-416	1864.99	0	4.25	0
6	[R].GNVIVVTFNYR.[V]	High	-	2	152-162	1281.70	0	3.75	0
7	[K].LSLFGDSIDIFK.[G]	High	-	2	38-49	1354.73	0	2.64	0
8	[K].LPLGSTEYPK.[L]	High	-	2	290-299	1104.60	0	2.73	0
9	[K].SANTYTYLFSQPSR.[M]	High	-	2	428-441	1634.78	0	4.62	0
10	[K].QDVTEEDFYK.[L]	High	-	2	355-364	1273.56	0	2.64	0

Description of Bovine Pancreatic BSAL peptide sequence prediction. The bold symbol represents the possible site of fixed and variable modifications. The confidence level for the identified protein was determined by the false discovery rate (FDR) and interpreted as high-confidence hits, which were 99% accurate. The charge is the sum of positive (basic) and negative (acidic) charge residues in neutral pH. Position in Master Protein is the location of the annotated predictive peptide sequence in the protein. Theo. MH+ [Da] is the protonated monoisotopic mass of the peptides in Daltons. Qvality q-value and Percolator q-Value Sequest HT are calculated from the number of target and decoy proteins and the minimum FDR is required for a hit to be considered correct (Qvality q-values and Percolator q-Value Sequest HT > 0.01 are considered high-confidence hits). XCorr Sequest HT indicates the number of fragment ions that are common to two different peptides with the same precursor mass and calculates the cross-correlation (XCorr) for all candidate peptides in search (XCorr value > 2 is considered favourable).

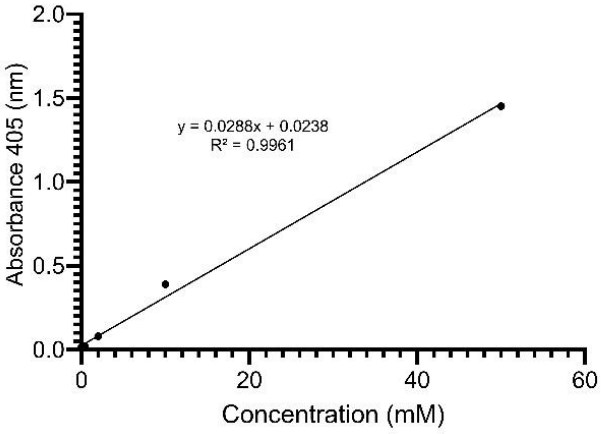

Figure 3. Standard curve of 4-nitrophenyl Palmitate as the substrate for the lipolytic enzyme assay. Absorbances at 405 nm were recorded at 25 °C. Each point represents the mean value of triplicates.

Table 2. Sequential Purification of Bovine Pancreatic BSAL

Purification stage	Volume (ml)	Protein Concentration A_{280}	Protein content (mg)	*Specific lipolytic activity (U/mg)	Total lipolytic activity (U)	Yield (%)
Aqueous crude pancreatic extract	200.0	57.30	11460.00	0.207 (0.1245)	2372.22	100.00
Anion-exchange chromatography (*Peak B*)	25.5	6.83	174.16	0.222 (0.1772)	38.66	1.63

Note: *Mean (SD). Specific lipolytic activity of porcine pancreatic lipase (1 mg/mL) = 0.223 (0.1913) U/mg. Extinction Coefficient = ~106230 $M^{-1}cm^{-1}$.

This postulated that there was very little loss of lipase during the delipidation process. Lipase activity was also compared using a commercially available porcine pancreatic lipase as a positive control. The concentration of the porcine pancreatic lipase was standardised to 1 mg/ml and yielded 0.0223 U/mg of the specific lipolytic activity. This suggests that the final purification of bovine BSAL is comparable to that of the porcine origin with specific BSAL lipolytic activity of 0.0222 U/mg. However, the total lipolytic activity of the aqueous crude extract was recorded higher based on the starting material volume and the protein concentration. Hence, the pancreatic bovine BSAL has a strong potential as a Halal enzyme replacement, specifically for pancreatic lipase.

DISCUSSION

This study employed a one-step purification of anion exchange chromatography, 1D SDS-PAGE, LC-MS/MS, and lipase activity assay to characterise bovine pancreatic BSAL. Previous work has reported the success of bovine pancreatic BSAL extraction and purification together with enzyme assay activity, N-terminal sequencing, and glycoprotein confirmation (Tanaka et al., 1999). The work presented herein successfully extracted and purified the bovine pancreatic BSAL and confirmed protein identification *via* LC-MS/MS with the enzyme assay activity compared against the established porcine lipase. Firstly, the bovine pancreatic crude extract was subjected to anion exchange chromatography, and a single peak was eluted at 10% NaCl concentration (peak B), which contained active BSAL lipolytic activities. The elution profiles of the BSAL enzyme are shown in Figure 1. A summary of the BSAL purification results is presented in Table 2. Fractions obtained from the one-step purification yielded higher BSAL lipolytic activity than the crude extract. Previously, pancreatic lipase was reported to have more significant similarity in molecular weight, similar amino-acid composition, and common antigenic determinants among mammalian lipases such as human, porcine, bovine, and ovine (Caro et al., 1981). On top of that, the specific lipase activity of bovine BSAL is comparable with that of porcine pancreatic lipase, which has been discovered in this study,

despite some arguments that lipases will have distinct lipolytic activity and specificity between different biological sources (Shahani et al., 1976). This discovery proves that purified bovine BSAL can be suggested as an alternative to porcine BSAL, considering that its specific lipolytic activity is similar to commercial pancreatic porcine lipase.

Bovine pancreatic BSAL is a glycoprotein with 597 amino acids and 65.12 kDa in molecular weight, as reported in the protein database system (PDB). In this study, the molecular weight of purified BSAL was determined by gel electrophoresis and confirmed with the LC-MS/MS. As shown in Figure 2, the purified product revealed a single band with approximately 60.0 kDa on the 1D SDS-PAGE and 65.12 kDa confirmed with the LC-MS/MS of the molecular weight. This corresponds to the previous finding that the molecular weight of purified bovine pancreatic BSAL ranged from 61.2 kDa to 65.5 kDa (Chen et al., 1998). In another study, bovine pancreatic BSAL was reported to have a molecular weight of 63.0 kDa as estimated by the SDS-PAGE (Iijima et al., 1998). This finding was also consistent with other research, whereby the 1D SDS-PAGE of the purified BSAL from defatted *Pyloric caeca* pancreatic tissue was seen as a homogenous band at 60.0 kDa (Gjellesvik et al., 1992). In contrast, the molecular weight of purified porcine pancreatic BSAL was reported to be slightly larger in the range of 70 to 85 kDa (Momsen & Brockman, 1977; Rudd et al., 1987). Meanwhile, the molecular weight of purified rat pancreatic BSAL was reported to be 70 kDa (Calame et al., 1975). Several mammalian BSALs were reported to have similar specificities and kinetic properties. However, the inconsistency of the physical properties and heterogeneity, including pI and molecular weight, have been controversial findings for BSAL from different animal sources (Rudd et al., 1987). It is speculated that although they may have similar protein cores, BSALs from different sources are subjected to varying degrees of modifications in various hosts, leading to molecular weight variations (Abouakil et al., 1988). Generally, glycans may contribute up to 30% of the molecular weight of glycoproteins (Puranik et al., 2022). Previously, the electrophoretic characterisation of the native bovine BSAL revealed the presence of N-linked sugars, which escalated the number of isoelectric forms and average pI, as well as the changes in the mass-to-charge ratio (Chen et al., 1998). Therefore, the expected size of the protein of interest is influenced by post-translational glycosylation modification. Alternatively, the variations of the C-terminal region of BSAL have also been reported to contribute towards differences in the molecular weight (Gjellesvik et al., 1992).

This work aims to identify the bovine pancreatic BSAL that could benefit pharmaceutical and medical applications, mainly to cater for Halal requirements. The LC-MS/MS proteomic analysis showed a positive protein identification, resulting in ten predictive peptide sequences showing 30.9% bovine pancreatic BSAL protein coverage to the same protein with the accession number P30122. It is difficult to compare the extent of proteome coverage in this study with previous studies that have focused on crystallisation (Chen et al., 1998) and N-terminal amino acid sequencing (Tanaka et al., 1999) of bovine pancreatic BSAL. Mass spectrometry helps detect the peptide as the amino acid sequence is unique to the candidate protein and provides better selectivity (Chiu et al., 2017; Rauh, 2012).

The existing literature on bovine BSAL purification is limited and relatively dated. The latest report on BSAL extraction and purification was two decades ago, reporting on the affinity binding sites of pancreatic BSAL in pancreatic and intestinal tissue of rats (Bruneau et al., 2000). Although there have been scientific reports on successful BSAL purification from bovine, this study presents a single-step purification of bovine pancreatic BSAL. Previously, bovine BSAL was isolated in a pure form by lyophilisation of fresh bovine pancreas, sucrose solution enzyme extraction, ammonium sulphate, and acetone fractional precipitation followed by gel filtration chromatography on Sephadex G-100 (Shahani et al., 1976). In another study by Tanaka et al. (1999), the bovine BSAL was purified from a defatted pancreas with 50 mM sodium phosphate pH 7.5 containing 0.5 mM PMSF and 2 mM benzamidine as an extraction buffer. Rudd et al. (1987) used different extraction buffers made up of 0.1 M sodium acetate buffer pH 4.8 containing 0.9% NaCl, 0.2% Triton X-100, 3 mM sodium taurocholate, 2 mM benzamide hydrochloride, 0.2 mM phenylmethylsulfonyl fluoride, 2mM hydrocinnamic acid, and 0.5 mM Nα-benzoyl-D, L-arginine was added with proteinase inhibitors were used during the BSAL extraction from frozen porcine pancreas. In the same study, the ammonium precipitation was done before running on Sephacryl S-200, followed by TSK 3000 SW columns to purify the porcine pancreatic BSAL (Rudd et al., 1987). The use of frozen pancreas was also observed in a study by Momsen & Brockman (1977), followed by Sephadex G-150 gel filtration, which is used to obtain purified porcine BSAL. Besides using fresh frozen pancreas as starting material, the pancreatic juice has been previously collected from dogs using a cannula, followed by ammonium precipitation, and applied to a DEAE-cellulose column and concentrated utilising a PM-10 membrane filter (Lee, 1978). However, the

cannulation process from the pancreatic duct for the pancreatic juices collection raises animal concerns, especially when using a non-invasive alternative (Steiner & Williams, 2002). A quick method using affinity chromatography of cholate-derivatized Sepharose successfully purified BSAL from bovine and porcine commercial crude and human milk whey has also been reported (Moore et al., 1996). Despite the various approaches, the findings are surprisingly consistent, with the molecular weight obtained for bovine pancreatic BSAL being approximately 60kDa. In summary, various purification methods are available, as well as the choice of starting material, extraction buffer, and columns used to purify pancreatic BSAL successfully. However, the study herein offers a more straightforward approach with minimal purification steps and directly using fresh bovine pancreas that would have gone to waste as part of the slaughtering process. This results in an improved yield of BSAL with the specific lipolytic activity of purified bovine BSAL comparable to commercial porcine pancreatic lipase.

CONCLUSION

BSAL was successfully purified and characterised from *Bos taurus* fresh pancreatic tissue. The BSAL yielded the highest after the anion exchange chromatography and was comparable to porcine lipase. Overall, the results of this study indicate that LC-MS/MS analysis followed by peptide prediction allows rapid identification of peptides with bovine pancreatic BSAL. This enables the extraction and purification process to obtain purified BSAL to be carried out effectively. Therefore, further studies on the current topic are recommended to determine the properties of bovine BSAL as a possible alternative for porcine lipase.

ACKNOWLEDGEMENT

Authors acknowledge the Ministry of Higher Education for funding this study via the Fundamental Research Grant Scheme (FRGS/1/2018/STG04/UITM/02/6). The authors thank the Faculty of Applied Sciences, Universiti Teknologi MARA and the Malaysia Genome and Vaccine Institute for laboratory facilities. The authors are also grateful to the Abattoir Complex, Department of Veterinary Services, Malaysia, for access to fresh bovine pancreas. Finally, the authors thank Miss Farahayu Binti Khairuddin from the Malaysia Genome and Vaccine Institute for technical support during the lipase activity measurement.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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